ADVANCES IN CARBOHYDRATE CHEMISTRY

VOLUME 4

ADVANCES IN CARBOHYDRATE CHEMISTRY

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(α-D-Glucopyranosyl-β-D-Fructofuranoside)

By Irving Levi and Clifford B. Purves

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I. Introduction

Sucrose is by far the most widespread, frequently the most abundant, sugar present in the sap of plants, and has been isolated, often in crude crystalline form, from the richer saps since the dawn of history.[1,2] Sugar cane has been a major commercial source for centuries, but the rise of the sugar beet industry to a comparable status commenced in 1797, when Achard announced that sugar could be extracted from beets on a commercial scale.[3] For many decades cane sugar and, more recently, beet sugar have been the outstanding examples of a very cheap, highly crystalline, organic compound produced in a state of unexcelled purity on a scale of tens of millions of tons a year. When this fact is considered,

(1) A. Chapman and V. W. Chapman, Article on Sugar in "Encyclopedia Britannica," Cambridge, Univ. Press, 11th ed., Vol. **26**, p. 32 (1911).

(2) E. O. Von Lippmann, "Die Chemie der Zuckerarten," Vieweg and Sohn, Braunschweig, 2nd ed., pp. 588–596 (1895). (A list of species examined up to 1895, usually by extracting the plant with aqueous alcohol and precipitating any sucrose from the extract as the difficultly soluble strontium saccharate.)

(3) G. Fairrie, "Sugar," Clay and Sons, Bungay, England, 1st ed., p. 24 (1925).

it is interesting to note that the melting point of pure sucrose, given as 180° in 1838 by Peligot,[4] and as 160–161° by Berzelius[5] in 1839, was definitely established only in the last twelve years. Bourne[6] considered that the proper value was 185–186°, and not 160°, as commonly quoted in textbooks. A later opinion that the melting point varied with the rate of heating[7] was invalidated in 1936, when it was found[8] that most previous determinations were lower than the correct value of 188° because minute traces of solvent or impurities brought about partial decomposition during the determination. Such was probably the cause of the difference between the "saccharose A," melting point 171°, and the "saccharose B," melting point 185°, described by Pictet[9] as being prepared by recrystallization from methanol and ethanol, respectively. On the other hand, Pictet's claim that sucrose octaacetate is polymorphous has been supported by other workers,[10–14] who have shown that a more labile form of melting point 69° changes on contact with water, ether and other liquids into the more stable allotrope of melting point 89°, which has a different crystal structure but the same specific rotation in solution.

Hassid, Doudoroff and Barker[15] recently synthesized sucrose, but this outstanding success was obtained by biochemical methods that threw little fresh light upon the constitution of the sugar. A direct proof of structure by an unambiguous chemical synthesis is still lacking. It is the purpose of this review to show that, in spite of this lack, other lines of evidence are concordant enough, and strong enough, to reveal the structure in a convincing way. The scientific literature concerning sucrose is enormous, for the sugar has served for a century as a convenient substrate for very many researches in enzyme, biological and

(4) E. Peligot, *Ann. chim. phys.*, **67**, 113 (1838); *J. prakt. Chem.*, **15**, 65 (1838); a comprehensive review of sugar chemistry.

(5) J. Berzelius, *Poggendorff's Ann.*, **47**, 289 (1839).

(6) B. Bourne, *Chem. News*, **110**, 47 (1914).

(7) K. Sandera and A. Mircev, *Z. Zuckerind. čechoslovak. Rep.*, **59**, 204 (1935); *Chem. Abstracts*, **29**, 2011, 4615 (1935).

(8) S. V. Shah and Y. M. Chakradeo, *Current Sci.*, **4**, 652 (1936); *Chem. Abstracts*, **30**, 4710 (1936).

(9) A. Pictet, *Helv. Chim. Acta*, **13**, 698 (1930).

(10) L. Duparc and R. Galopin, *Helv. Chim. Acta.* **13**, 702 (1930).

(11) M. Frèrejacque, *Compt. rend.* **503**, 731 (1936).

(12) K. Sandera, *Chem. Listy*, **33**, 139 (1939); *Chem. Abstracts*, **33**, 6252 (1939). This article quotes earlier melting points and specific rotations.

(13) R. P. Linstead, A. Rutenberg, W. G. Dauben and W. L. Evans, *J. Am. Chem. Soc.*, **62**, 3260 (1940).

(14) C. D. West, *J. Am. Chem. Soc.*, **63**, 630 (1941).

(15) W. Z. Hassid, M. Doudoroff and H. A. Barker, *J. Am. Chem. Soc.*, **66**, 1416 (1944); *Science*, **100**, 51 (1944).

physical chemistry. A massive literature concerns the details of its physical properties and its large-scale production. All these subjects are omitted from this review, except in so far as they contribute information concerning the chemical structure and configuration of the sugar.

II. Earlier Observations on Structure

1. *Determination of the Sucrose–Invert Sugar Relationship*

Precise knowledge of the chemistry of sucrose and glucose began with the establishment of their elementary composition by Lavoisier, Gay-Lussac, Thenard, Dumas, Crum, de Saussure and others. The analyses by Prout[16] were particularly interesting although not exact. Prout showed that combustion of sucrose and glucose in oxygen produced gases equal in volume to the oxygen consumed. Knowing that the combustion of the carbon to carbon dioxide caused no change in the gas volume, he inferred that the two sugars contained oxygen and hydrogen in the ratio characteristic of water. Reviews by Liebig,[17] Peligot[4] and Berzelius[5] summarized the analyses and assigned compositions to sucrose and glucose similar to those accepted today. At that time the equivalent weights of sugars were estimated by a method originated in 1815 by Berzelius, who analyzed the saccharates formed with inorganic hydroxides such as those of lead, the alkaline earths and the alkali metals, or the crystalline double compounds of sodium chloride with sucrose and glucose. The results obtained for sucrose, principally by Peligot[4] and Soubeiran,[18] suggested formulas based on C_{12} units or on even, integral multiples thereof, the exact formula depending on the atomic weights accepted for carbon and oxygen and on the constitution assumed for the saccharates. Such formulas fitted views then current concerning the fermentation of sucrose, which had been studied quantitatively by Gay-Lussac[19] and was known to produce 51.3 and 53.7 parts by weight of carbon dioxide and ethanol, respectively. One mole of sucrose had also been shown to utilize one mole of water during the fermentation.[20] According to Liebig,[17] alcohol ($C_4H_{12}O_2$) was the hydrate of diethyl ether ($C_4H_{10}O$). Sucrose ($C_{12}H_{22}O_{11}$) was a compound of four "atoms" of carbon dioxide, two "atoms" of ether and one "atom" of water of crystallization, the latter being replaceable by bases like lead oxide. The formula of glucose monohydrate was written $C_{12}H_{28}O_{14}$ to show it as a more hydrated form of sucrose.

(16) W. Prout, *Phil. Trans.*, **117**, 355 (1827).
(17) J. Liebig, *Poggendorff's Ann.*, **31**, 321 (1834).
(18) E. Soubeiran, *Ann.*, **43**, 223 (1842).
(19) L. J. Gay-Lussac, *Ann. chim. phys.*, [1] **76**, 245 (1810).
(20) J. B. Dumas and P. Boullay, fils, *Ann. chim. phys.*, [2] **37**, 45 (1828).

It was known at an early date that grape sugar, the sugar isolated from diabetic urine[4,21] and the sugar from the acid hydrolysis of starch were identical. The identity, however, was often and more or less tacitly extended to include as "glucose" uncrystallized sirups with the same elementary composition and general properties.[22] This confusing tendency was repeatedly deprecated by Biot,[23] who knew that crystalline glucose rotated plane polarized light to the right, whereas the "uncrystallized glucose" (invert sugar) derived from sucrose by acid hydrolysis was levorotatory.[24] Moreover, Peligot[4] pointed out that crystalline glucose gave no color with concentrated hydrochloric acid, but that sucrose, both before and after acid hydrolysis, did so. The same author was aware that the residue from an incomplete fermentation of sucrose was levorotatory, Persoz being credited with the initial observation both by Berthelot[25] and by Pasteur.[26]

The full significance of these observations was brought out in the period 1846 to 1856 by Dubrunfaut,[27] who established or confirmed the following facts by direct experiment. Pure sucrose, when carefully hydrolyzed by weak acids, yielded 105 parts of invert sugar, which was weighed after drying at 100° *in vacuo*. Contrary to the current opinion, invert sugar was not transformed to glucose by crystallization; although a certain proportion of well-characterized glucose might separate, the mother sirup became more levorotatory and, therefore, had to contain a different sugar. The same inference was drawn from the fact that, when invert sugar was fermented by yeast, and particularly in a lactic acid fermentation, the glucose was consumed preferentially, and strongly levorotatory sirups were obtained by interrupting the fermentations. When milk of lime was added to a 10% aqueous solution of invert sugar and the resulting, sparingly soluble, crystalline basic compound was recovered and decomposed with oxalic acid, the new constituent was isolated as a practically pure sirup. This sirup, carefully dried, had the same elementary composition as invert sugar or glucose and yielded the same amounts of alcohol and carbon dioxide when fermented by yeast. The specific rotatory power, about four times more levorotatory than that of invert sugar, confirmed the identity of the new sugar (which he simply

(21) L. Pasteur, *Ann. chim. phys.*, [3] **31**, 67 (1851).

(22) For example, L. J. Thenard, L. J. Gay-Lussac, J. B. Biot and J. B. Dumas Report to the French Academy of Sciences, *Compt. rend.*, **7**, 106 (1838).

(23) For example, J. B. Biot, *Compt. rend.*, **15**, 619, 693 (1842).

(24) J. B. Biot, *Compt. rend.* **17**, 755 (1843).

(25) M. Berthelot, *Compt. rend.*, **50**, 980 (1860).

(26) L. Pasteur, *Ann. chim. phys.*, [3] **58**, 323 (1860); an extensive literature review is included.

(27) A.-P. Dubrunfaut, *Compt. rend.*, **25**, 307 (1847); **29**, 51 (1849); **42**, 901 (1856).

termed "sucre liquide") with that obtained by hydrolyzing the polysaccharide inulin. Moreover, the magnitude of the levorotation decreased markedly with increase of temperature and at twice the rate displayed by the rotation of invert sugar. The composition, optical rotation and rotation change with temperature peculiar to the latter were duplicated by a synthetic mixture of equal parts of crystalline glucose and the new levorotatory amorphous sugar, his "sucre liquide." This sugar, the discovery of which is one of the great services that Dubrunfaut rendered to science, later received the name "levulose" from Berthelot and eventually the present scientific name "D-fructose" from Fischer.[28] Dubrunfaut summarized his results in the equation:

$$\underset{\substack{\text{Sucre de canne}\\ [\alpha]_j\,=\,+73.8^\circ}}{2\,C_{12}H_{11}O_{11}} + 2\,HO \rightarrow \underset{\substack{\text{Glucose}\\ [\alpha]_j\,=\,+53.2^\circ}}{C_{12}H_{12}O_{12}} + \underset{\substack{\text{Sucre liquide}\\ [\alpha]_j\,=\,-106^\circ}}{C_{12}H_{12}O_{12}}$$

in which the atomic weights of carbon and oxygen were taken as 6 and 8, respectively.

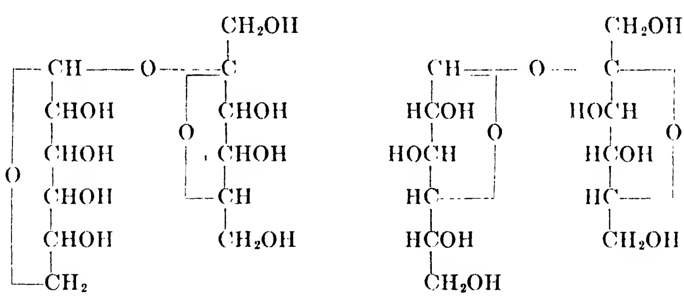
Modern atomic weights gained acceptance in the 1860's.[29] About twenty years later Raoult[30] determined the molecular weight of sucrose as 342, and that of invert sugar as 180, by noting the freezing points of their dilute aqueous solutions. Kiliani,[31] employing the cyanohydrin synthesis, then showed conclusively that D-glucose was a six-carbon aldose, a result that was quickly supported by the new physical method.[32,33] Levulose, or D-fructose, known for years as the "uncrystallizable sugar," was finally crystallized by Jungfleisch and Lefranc,[34] whose success made it easy to confirm the elementary composition of the sugar,[27,35–37] determine its molecular weight[32] and recognize it as a six-carbon 2-ketose.[38] By 1890, Dubrunfaut's fundamental equation for the cleavage of sucrose became generally accepted in the familiar form:

$$\underset{\text{Sucrose}}{C_{12}H_{22}O_{11}} + H_2O \rightarrow \underset{\text{D-Glucose}}{C_6H_{12}O_6} + \underset{\text{D-Fructose}}{C_6H_{12}O_6}$$

(28) E. Fischer, *Ber.*, **23,** 930 (1890).
(29) C. Graebe, "Geschichte der organischen Chemie," J. Springer, Berlin, pp. 223–33 (1920).
(30) F. M. Raoult, *Compt. rend.*, **94,** 1517 (1882).
(31) H. Kiliani, *Ber.*, **19,** 1128 (1886).
(32) H. T. Brown and G. H. Morris, *Chem. News*, **57,** 196 (1888).
(33) B. Tollens and F. Mayer, *Ber.*, **21,** 1566 (1888).
(34) E. Jungfleisch and E. Lefranc, *Compt. rend.*, **93,** 547 (1881).
(35) A. Herzfeld, *Ann.*, **244,** 274 (1888); a review of the literature is included.
(36) H. Winter, *Ann.*, **244,** 295 (1888).
(37) M. Hönig and S. Schubert, *Monatsh.*, **8,** 529 (1887).
(38) H. Kiliani, *Ber.*, **19,** 221 (1886).

2. *Suggested Constitutional Formulas*

The expansion of these molecular formulas into satisfactory constitutional formulas was intimately linked with the growth of knowledge concerning the optical mutarotation of crystalline D-glucose freshly dissolved in water. Dubrunfaut[39] was apparently the first to record that the initial specific rotation of D-glucose monohydrate in water was nearly twice the final value, which was reached only after the solution had stood for some hours or had been heated. Dubrunfaut made the observation while developing a polarimetric method for estimating sucrose and invert sugar. A few years later, Pasteur[40] observed in detail the change with time shown by the rotation of a 10% solution of the crystalline D-glucose-sodium chloride compound. According to reviews by Brown and Pickering[41] and by Von Lippmann,[42] such mutarotations were attributed to the establishment of equilibria in solution between a colloidal crystal aggregate and a molecularly dispersed state, or between a crystalline and an amorphous form, a hydrated and a solvated form, or to the formation and hydrolysis of an ethylene oxide ring. The speculation which eventually proved correct was made by Tollens,[43] who considered that the mutarotation of D-glucose and D-fructose, as well as some abnormal properties of their carbonyl groups, was to be explained by their existence as unstable cyclic hemiacetals. By analogy, Tollens assigned the mixed cyclic acetal structure I to the nonreducing sucrose molecule, although the exact positions of the acetal rings were left undecided. Such a formula agreed with the existence of a presumed



I
Sucrose (Tollens, 1883)

II
Sucrose (Fischer, 1893)

(39) A.-P. Dubrunfaut, *Compt. rend.*, **23**, 38 (1846); *Ann. chim. phys.*, [3] **18**, 99 (1846).

(40) Ref. 21, p. 95. Although Pasteur did not realize it at that early time, all of his observations except the first fit a first-order equation quite well.

(41) H. T. Brown and S. U. Pickering, *J. Chem. Soc.*, **71**, 756 (1897).

(42) Ref. 2, pp. 125–30.

(43) B. Tollens, *Ber.*, **16**, 921 (1883).

octaacetate[44] and a presumed octanitrate[45–48] of sucrose, although these fully substituted derivatives were not crystallized and reliably analyzed until 1887[49] and 1919,[50] respectively. A recent review by Hudson[51] commemorates Emil Fischer's classic work that led to the assignment of stereochemical configurations to D-glucose and D-fructose. These configurations are included in the Fischer formula II for sucrose, together with his suggestion that the cyclic structure was five membered in both halves of the molecule. This suggestion was based in part upon Baeyer's strain theory,[52] which stated that five-membered rings formed most readily, and in part upon a previous assumption that the sugar lactones contained rings of that size.[53]

Consideration of a structure such as II shows that both the D-glucoside and D-fructoside portions may have an alpha or a beta configuration about the glycosidic carbon atoms and that four isomers are therefore possible. The line of thought that eventually identified sucrose with the proper isomer commenced about 1830, when Dubrunfaut, as stated by Pasteur,[26] observed that the first action of aqueous yeast suspensions was to invert the sugar. Sucrose was fermented more slowly than D-glucose.[54] Berthelot[25] later succeeded in separating the inverting from the fermenting action by extracting the macerated yeast with water. Alcohol when mixed with the extract precipitated a yellow nitrogenous mass that retained the capacity to invert sucrose and that resembled protein in being coagulated by heat or nitric acid. O'Sullivan and Tompson[55] reviewed subsequent improvements up to 1890 in the preparation of the active agent, "invertin,"[56] or invertase,[55] and have also to be credited with an admirable preliminary study of its mode of action. They noted the freezing point of a dilute, invertase-containing sucrose solution at various times and followed the decrease in molecular weight from that of sucrose (342) to that of invert sugar (180) as inversion proceeded. Knowing by this means the fraction of sucrose inverted at any time, comparison with corresponding values calculated from the polarimetric

(44) A. Herzfeld, *Ber.*, **13**, 265 (1880).
(45) C. F. Schoenbein, *Poggendorff's Ann.*, **70**, 100 (1847).
(46) A. Sobrero, *Compt. rend.*, **24**, 247 (1847).
(47) A. H. Elliott, *J. Am. Chem. Soc.*, **4**, 147 (1882).
(48) W. Will and F. Lenze, *Ber.*, **31**, 68 (1898).
(49) A. Herzfeld, *Z. Ver. deut. Zucker-Ind.*, **37**, 422 (1887), quoted from ref. 14.
(50) E. J. Hoffman and V. P. Hawse, *J. Am. Chem. Soc.*, **41**, 235 (1919).
(51) C. S. Hudson, *J. Chem. Education*, **18**, 353 (1941).
(52) A. Baeyer, *Ber.*, **18**, 2267 (1885).
(53) E. Fischer, *Ber.*, **26**, 2400 (1893).
(54) H. Rose, *J. prakt. Chem.*, **23**, 393 (1841).
(55) C. O'Sullivan and F. W. Tompson, *J. Chem. Soc.*, **57**, 834 (1890).
(56) E. Donat, *Ber.*, **8**, 795 (1875).

changes showed that the latter gave a misleading underestimate of its extent. When the inversion was arrested, and the invertase inactivated, instantaneously by adding the minimum amount of alkali, the rotation swiftly decreased to a final constant value which was characteristic of the particular degree of inversion, and which was concordant with the molecular weight determinations. The results showed that the cleavage of sucrose by invertase followed a monomolecular course but that the cleavage products were liberated in an unstable, more dextrorotatory, state. These products promptly assumed their final rotations when their "bi-rotation" was catalyzed by the added alkali. Since the spontaneous mutarotation of ordinary D-fructose was known to be small and swift, it was surmised that the "bi-rotation" was that of freshly liberated α-D-glucose and that sucrose was an α-D-rather than a β-D-glucoside. The above information made it possible to study the influence of temperature and hydrogen ion concentration on invertase in a quantitative way. The fact that the inversion obeyed the first-order rate equation for simple organic reactions also tended to invalidate the controversial supposition[26] that yeasts and enzymes could not be expected to observe such physical laws because their reactions were governed by vital forces. With the exception of Armstrong,[57] later workers overlooked the systematic error in the polarimetric method of following the inversion by invertase until Hudson[58] redirected attention to the correct observations of O'Sullivan and Tompson. It is interesting to note that Barth's[59] data showed as early as 1878 that inversion by invertase obeyed the equation for a first-order reaction over the range from 3% to 70 or 75% of completion. Although unrecognized at the time, Barth's success arose from the fact that he followed the inversion by the increase in the alkaline-copper-reducing power of the solution. This method of estimation is obviously insensitive to mutarotation effects.

Wilhelmy[60] was one of several who studied the polarimetric changes occurring when sucrose was hydrolyzed by acids. His articles, which are now classics in physical chemistry thanks largely to Ostwald's[61] early recognition of their importance, developed the mathematical expressions to describe the progress of a unimolecular reaction. They showed that the inversion of sucrose by several dilute acids obeyed the equation, although the extent of inversion was calculated directly from the observed polarimetric readings. Other investigators cast doubt upon the latter claim until Hudson[62] pointed out in a review that the mutarotations of

(57) E. F. Armstrong, *J. Chem. Soc.*, **83**, 1305 (1903).
(58) C. S. Hudson, *J. Am. Chem. Soc.*, **30**, 1160 (1908).
(59) M. Barth, *Ber.*, **11**, 474 (1878).
(60) L. Wilhelmy, *Poggendorff's Ann.*, **81**, 413, 499 (1850).
(61) W. Ostwald, *J. prakt. Chem.*, **29**, 385 (1884).
(62) C. S. Hudson, *J. Am. Chem. Soc.*, **32**, 885 (1910).

D-glucose and D-fructose were practically instantaneous in the acid solutions that Wilhelmy used and could produce no appreciable time lag in his measurements. Although this circumstance has, until now, made inversion by acids of little value as a tool for determining the glycosidic configurations in sucrose, the precision and ease with which the reaction can be followed have made it invaluable for the study of hydrogen ion concentration, common ion and strong electrolyte effects, activation energy, analytical methods, and other matters of interest to physical chemists. Articles including reviews of this field are available.[63–68] The inversion, with or without invertase or acid, has also been useful in studying the chemical effect of the deuterium ion,[69–72] of high pressure,[73] of sonic and supersonic energy[74,75] of ultraviolet light[76–78] of high-frequency electric energy[79] and of variation in the dielectric constant of the solvent.[80]

III. Determination of the Cyclic Structures in Sucrose[81]

1. *The* D-*Glucopyranosyl Ring*

The discovery, by Purdie and his collaborators,[82] that the reaction between a lower alkyl iodide and the silver salt of a simple hydroxy acid yielded a proportion of the corresponding alkoxy ester, led to the silver

(63) E. O. Von Lippmann, Ref. 2, pp. 725–736.

(64) L. J. Heidt and C. B. Purves, *J. Am. Chem. Soc.*, **60**, 1206 (1938); **62**, 1006 (1940).

(65) A. Lamble and W. C. M. Lewis, *J. Chem. Soc.*, **107**, 233 (1915).

(66) G. Scatchard, *J. Am. Chem. Soc.*, **48**, 2259 (1926).

(67) D. I. Hitchcock and Ruth B. Dougan, *J. Phys. Chem.*, **39**, 1177 (1935).

(68) J. M. Sturtevant, *J. Am. Chem. Soc.*, **59**, 1528 (1937).

(69) E. W. R. Steacie, *Z. physik. Chem.*, **B27**, 6 (1934).

(70) P. Gross, H. Suess and H. Steiner, *Naturwissenschaften*, **22**, 662 (1934).

(71) E. A. Moelwyn-Hughes, *Z. physik. Chem.*, **B26**, 272 (1934).

(72) E. A. Moelwyn-Hughes and K. F. Bonhoeffer, *Naturwissenschaften*, **22**, 174 (1934).

(73) F. V. Sander, Jr., *J. Biol. Chem.*, **148**, 311 (1943).

(74) E. W. Flosdorf and L. A. Chambers, *J. Am. Chem. Soc.*, **55**, 3051 (1933).

(75) S. Sokolov, *Tech. Phys. U. S. S. R.*, **3**, 176 (1936); *Chem. Abstracts*, **31**, 4182 (1937).

(76) N. Taketomi and K. Miura, *J. Soc. Chem. Ind. Japan*, **33**, 99 (1930); *Chem. Abstracts*, **24**, 2942 (1930).

(77) A. Guillaume and G. Tanret, *Bull. soc. chim. biol.*, **18**, 556 (1936); *Chem. Abstracts*, **30**, 5503 (1936).

(78) L. J. Heidt, *J. Am. Chem. Soc.*, **61**, 3223 (1939).

(79) S. P. Voskresenskii and E. N. Malashenko, *J. Gen. Chem.* (*U. S. S. R.*), **9**, 1118 (1939); *Chem. Abstracts*, **33**, 8119 (1939).

(80) C. J. Plank and H. Hunt, *J. Phys. Chem.*, **45**, 1403 (1941).

(81) See also W. N. Haworth, "The Constitution of Sugars," Arnold and Co., London, pp. 17–33, 68–71 (1929).

(82) T. Purdie and W. Pitkeathly, *J. Chem. Soc.*, **75**, 153 (1899).

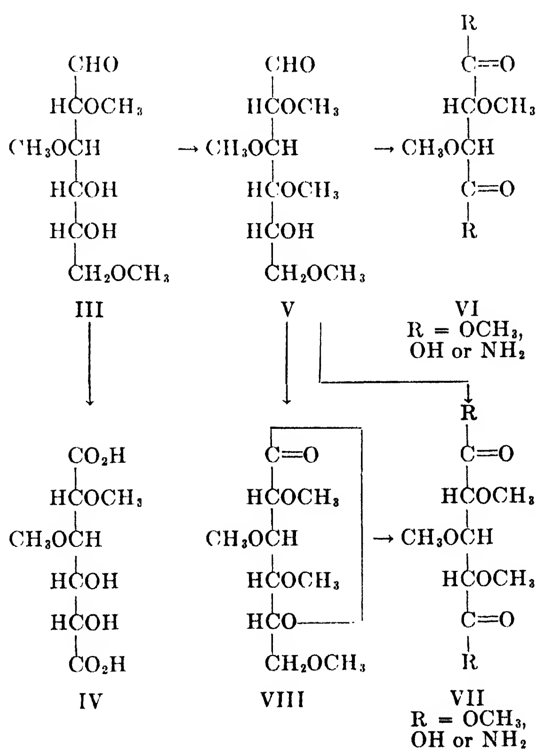
oxide–methyl iodide method of methylation. Purdie and Irvine[83] were the first to use the new procedure in the carbohydrate field and their well-known articles include the first description of octamethylsucrose. The preparation of this compound was greatly facilitated by Haworth's[84] observation that the methylation of sucrose with dimethyl sulfate and strong aqueous caustic soda yielded an uncrystallized "heptamethylsucrose" in one operation. Remethylation of the latter by the earlier method then gave octamethylsucrose as a slightly viscid, colorless liquid, boiling point 176° at 0.05 mm., with a dextrorotation of 69.3° in methanol.[85]

Later improvements in technique rendered such substances very readily accessible.[86,87] The existence of a homogeneous octamethylsucrose, together with that of an octapropionate,[88] confirmed Tollens' view[43] that only eight hydroxyl groups were present in the sucrose molecule. Moreover, since no molecular rearrangement has ever been found to occur during the methylation of nonreducing carbohydrates, the hydroxyl positions in sucrose were those occupied by methyl ether groups in octamethylsucrose. Since these groups are very stable, they retained their positions during the cleavage of the methylated sucrose to a tetramethyl-D-glucose and a tetramethyl-D-fructose. It followed that these two products would not have been methylated in positions originally shielded from methylation by the ring structures of sucrose.

Contrary to experience with the unsubstituted sugar, no optical inversion took place when the octamethyl derivative was completely hydrolyzed with hot, dilute mineral acid,[83] the overall decrease in specific rotation being[84] from +66.7° to +57.0°. After isolation, the portion of the product that crystallized was found to be identical with a tetramethyl-D-glucose, melting point 96° and $[\alpha]^{20}_{D}$ + 81.3° in water (final),[86] which could also be prepared by the complete methylation and subsequent hydrolysis of ordinary methyl D-glucoside.[83,85] The positions of the methyl ether groups in this tetramethyl-D-glucose were established by several researches, of which the first, by Irvine and Hirst,[89] showed that a certain trimethyl-D-glucose (III) could be oxidized to a *dimethyl*saccharic acid (IV). The loss of one substituent without the simultaneous cleavage of the hexose chain was clear evidence that the missing unit had occupied the sixth or terminal position in the trimethyl-D-glucose. The same

(83) T. Purdie and J. C. Irvine, *J. Chem. Soc.*, **83**, 1021 (1903); **87**, 1022 (1905).
(84) W. N. Haworth, *J. Chem. Soc.*, (*a*) **107**, 8 (1915); (*b*) **117**, 199 (1920).
(85) W. N. Haworth and J. Law, *J. Chem. Soc.*, **109**, 1314 (1916).
(86) E. S. West and R. F. Holden, *J. Am. Chem. Soc.*, **56**, 930 (1934).
(87) E. Pacsu and S. M. Trister, *J. Am. Chem. Soc.*, **61**, 2442 (1939).
(88) C. D. Hurd and K. M. Gordon, *J. Am. Chem. Soc.*, **63**, 2657 (1941).
(89) J. C. Irvine and E. L. Hirst, *J. Chem. Soc.*, **121**, 1213 (1922).

conclusion was true of the tetramethyl-D-glucose (V), because it was readily obtained by the further methylation of the trimethyl derivative. The oxidation of the tetramethyl-D-glucose itself was then undertaken by Hirst,[90] who employed nitric acid of density 1.42, first at room temperature and finally at 90°. After forming the methyl ester of the acidic product, distillation yielded a main fraction corresponding in properties to a 60:40 mixture of a dimethyl dimethoxysuccinate (VI, R = OCH_3) and a dimethyl trimethoxyglutarate (VII, R = OCH_3). The constituents of the mixture were separated in pure form as the crystalline, sparingly soluble diamides. One of those, identified as the diamide of internally compensated, optically inactive *xylo*-trimethoxyglutaric acid, (VII, R = OH), accounted for 15 to 20% of the tetramethyl-D-glucose, and the other, the dextrorotatory (L-*threo*)-dimethoxysuccinamide, (VI, R = NH_2), for 25 to 40%. Although it was true that none of VII was isolated unless the oxidizing conditions were favorable, the fact that it arose at all was proof that three adjacent positions in the tetramethyl-D-glucose were methylated. These positions were the second, third and



(90) E. L. Hirst, *J. Chem. Soc.*, 350 (1926).

fourth, as shown in V, because the alternative 3,4,5 sequence would have yielded the optically active (D-*arabo*)-trimethoxyglutaric acid, known to be optically stable in the conditions of the experiment. Using Irvine's system of nomenclature[91] the tetramethyl-D-glucose was indexed[90,92] as the 2,3,4,6 isomer. A simultaneous and independent proof of this conclusion was furnished by Charlton, Haworth and Peat,[92] when they demonstrated that the tetramethyl-D-gluconolactone VIII, in aqueous solution at room temperature, came into equilibrium with the free acid within one day. This behavior was shown to be characteristic of several other fully methylated sugar lactones whose 1,5 cyclic structures were known from the results of nitric acid oxidations and from other kinds of evidence. On the other hand, aqueous solutions of all similar lactones of 1,4 structure failed to come into equilibrium with the free acids within a week under the same conditions. The validity of those generalizations in the present case was checked by oxidizing VIII with nitric acid in about 40% yield to VII.[93]

2. *The* D-*Fructofuranosyl Ring*

Although tetramethyl-D-glucose crystallized readily from the hydrolysis product of octamethylsucrose, the isolation in relatively pure form of the methylated D-fructose moiety was a matter of difficulty[83] and was accomplished only when "heptamethylsucrose" was hydrolyzed.[84b] The latter preparation was quickly recognized as a mixture[94,95] but, nevertheless, acid hydrolysis yielded for the most part various trimethyl-D-glucoses from which the more volatile tetramethyl-D-fructose was readily separated by fractional distillation.[94,96] Traces of methylated D-glucoses were removed from the crude product by selective oxidation with bromine water,[97] this method appearing preferable to a selective oxidation with alkaline hypoiodite[98] or to a selective condensation of the tetramethyl-D-fructose with methanolic hydrogen chloride.[95] The pure, liquid tetramethyl-D-fructose,[97] boiling point 110–112° at 0.35 mm., n^{13}D 1.4513, had a final specific rotation in water of $[\alpha]^{16}$D +31.3°. A value of about +30° was calculated from the specific rotation of +57° found for hydrolyzed octamethylsucrose, the assumption being made that this product

(91) J. C. Irvine, *Proc. Chem. Soc.*, **29**, 69 (1913).

(92) W. Charlton, W. N. Haworth and S. Peat, *J. Chem. Soc.*, 89 (1926).

(93) W. N. Haworth, E. L. Hirst and E. J. Miller, *J. Chem. Soc.*, 2436 (1927).

(94) W. N. Haworth and W. G. Sedgwick, *J. Chem. Soc.*, 2573 (1926).

(95) J. C. Irvine and E. T. Stiller, *J. Am. Chem. Soc.*, **54**, 1486 (1932).

(96) H. C. Carrington, W. N. Haworth and E. L. Hirst, *J. Am. Chem. Soc.*, **55**, 1084 (1933).

(97) W. N. Haworth, E. L. Hirst and V. S. Nicholson, *J. Chem. Soc.*, 1513 (1927).

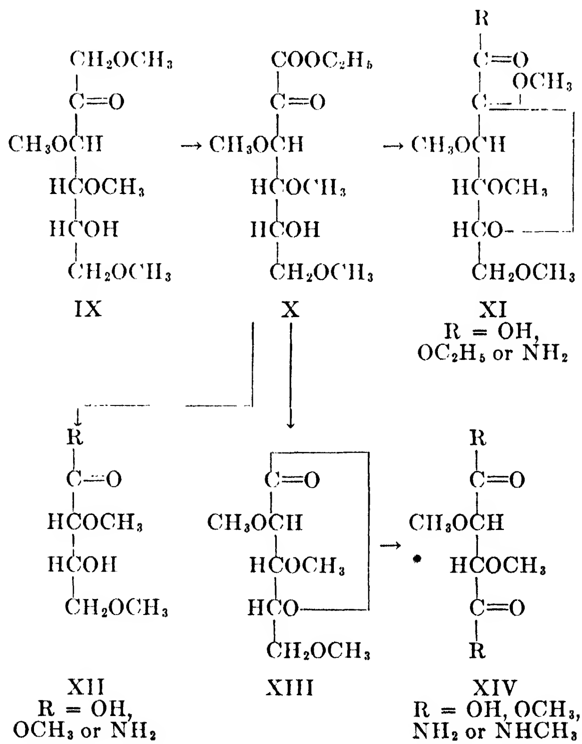
(98) G. McOwan, *J. Chem. Soc.*, 1737 (1926).

consisted of the tetramethyl-D-fructose and the tetramethyl-D-glucose of rotation +81.3° in equimolar amounts.[85] The agreement made it highly unlikely that any gross rearrangement had occurred in the hydrolysis of the methylated sucrose or in the isolation of the tetramethyl-D-fructose. Moreover, the dextrorotation of 31.3° found for the latter made it quite clear that the ring structure of the D-fructose half of sucrose was not the same as that present in the normal methyl β-D-fructoside of Hudson and Brauns,[99] which yielded the levorotatory crystalline tetramethyl-D-fructose[100] of melting point 98–99° and $[\alpha]_D$ −85.6°, that had originally been discovered by Purdie and Paul.[101] The same dextrorotatory tetramethyl-D-fructose was derived from inulin[102] and from the "γ-methylfructoside" mixture.[103] It followed that the inversion of unsubstituted sucrose, inulin or "γ-methylfructoside," either by acids or by invertase, involved a ring shift of the D-fructose units from the same "γ" to the normal form.[85,104]

Haworth, Hirst and their collaborators[97,105,106] carried out the oxidation of the tetramethyl-D-fructose IX obtained from sucrose, with nitric acid (density 1.42) at temperatures rising to 93° and the main product was isolated in about 60% yield as the volatile, copper-reducing ethyl ester X. Methylation of the latter gave the corresponding liquid methyl glycoside mixture (XI, R = OC_2H_5), one of whose amides (XI, R = NH_2) was fortunately obtained pure and crystalline (melting point 99–100°; overall yield from tetramethyl fructose, about 40%). The same amide was also prepared from the methyl ester of XI and its constitution clearly showed that one of the methoxy groups occupied a terminal position in the tetramethyl-D-fructose (IX). When X was oxidized with alkaline permanganate, there resulted a 30% yield of a hydroxydimethoxybutyric acid (XII, R = OH), which was isolated as a liquid methyl ester and as a crystalline amide (XII, R = NH_2). The failure of the latter to react positively in the Weerman hypochlorite test[107] for α-hydroxyamides suggested that a methoxy group occupied the alpha position, and the sluggishness with which the free acid was oxidized by nitric acid led to the tentative assignment of the other methoxy group to the omega

(99) C. S. Hudson and D. H. Brauns, *J. Am. Chem. Soc.*, **38,** 1216 (1916).
(100) Ettie S. Steele, *J. Chem. Soc.*, **113,** 257 (1918).
(101) T. Purdie and D. M. Paul, *J. Chem. Soc.*, **91,** 289 (1907).
(102) J. C. Irvine, Ettie S. Steele and Mary I. Shannon, *J. Chem. Soc.*, **121,** 1060 (1922).
(103) R. C. Menzies, *J. Chem. Soc.*, **121,** 2238 (1922).
(104) J. C. Irvine and G. Robertson, *J. Chem. Soc.*, **109,** 1305 (1916).
(105) W. N. Haworth and E. L. Hirst, *J. Chem. Soc.*, 1858 (1926).
(106) J. Avery, W. N. Haworth and E. L. Hirst, *J. Chem. Soc.*, 2308 (1927).
(107) R. A. Weerman, *Rec. trav. chim.*, **37,** 16 (1918).

position. This negative evidence was confirmed by a carefully limited, acid permanganate oxidation of X at room temperature to trimethyl-D-arabono-γ-lactone (XIII), which was isolated in about 50% yield and whose L-isomer was already known. The isolation of this substance proved that the terminal methoxy group lost in the initial oxidation of IX to X was in the first, and not the sixth, position. A later article[108] describes the oxidation of the D-lactone XIII with nitric acid to levorotatory (D-*threo*)-dimethoxysuccinic acid (XIV), recovered as the dimethyl ester, as the amide with melting point 270°, and as the *N*-methylamide



IX; X; XI, R = OH, OC_2H_5 or NH_2; XII, R = OH, OCH_3 or NH_2; XIII; XIV, R = OH, OCH_3, NH_2 or $NHCH_3$

with melting point 205°, in about 35% of the theoretical yield. As might be expected of a 1,4 lactone, the arabonolactone XIII was of the stable type that hydrolyzed slowly in water. All these results identified the tetramethyl-D-fructose IX as the 1,3,4,6 variety and excluded any other possibility. This conclusion was fortified by indirect evidence concerning the behavior of the isomeric 1,3,4,5-tetramethyl-D-fructose which, on oxidation with nitric acid, eventually yielded a proportion of the

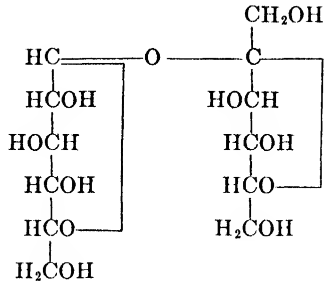
(108) W. N. Haworth, E. L. Hirst and A. Learner, *J. Chem. Soc.*, 2432 (1927).

optically active (D-*arabo*)-trimethoxyglutaric acid, as well as *i*-dimethoxysuccinic acid.[109]

The above outline does scant justice to the technical and psychological difficulties overcome by the pioneer investigators of the 1,3,4,6-tetramethyl-D-fructose. Failure to crystallize made the purification of the substance somewhat uncertain, and it was found to be partly changed to 5-methoxymethylfurfural during hydrolyses with dilute aqueous acid, degradation with nitric acid or acetylation with sodium acetate and acetic anhydride.[97] The ready formation of furfural derivatives from 1,4 (or 2,5) cyclic sugars in general explained their ability to decolorize ice-cold, dilute potassium permanganate solution.[110] Previous work had shown that this early test to differentiate normal from "γ" sugar derivatives depended on the presence of an adventitious impurity.[111] After the oxidation of the tetramethyl-D-fructose with nitric acid, the acidic product, still containing traces of nitric acid, was customarily dried by the continuous addition and distillation of absolute ethanol. It was not recognized for some years that the uncrystallized products isolated by fractional distillation were in part ethyl esters formed in the isolation, instead of the expected lactones. It was also tacitly assumed that the normal cyclic structure of D-fructose was 2,5 and would lead on methylation to 1,3,4,6-tetramethyl-D-fructose. The derivative isolated from octamethylsucrose would on this erroneous assumption have to be an isomer. In these circumstances, great hesitation was apparent in the interpretation of the analytical data, the tetramethyl-D-fructose from sucrose being cautiously held at various times to be unsubstituted in the 2,3,[85] 2,3 or 2,4,[111] 2,4,[98,112] 2,5 or 2,6[113] and 2,6[114,115] positions. Convincing evidence that the tetramethyl-D-fructose was in reality the 1,3,4,6 isomer, unsubstituted in the 2,5 positions, was forthcoming only when the investigation was based upon the analyses and rigorous identification of pure crystalline derivatives, such as lactones or amides, instead of upon sirups.[105] Adopting the nomenclature of Goodyear and Haworth,[116,117] the 2,5 ring structure of the 1,3,4,6-tetramethyl-D-fructose was five-membered or furan; the 1,5 six-membered ring in 2,3,4,6-tetramethyl-D-glucose was pyran, and sucrose (XV) was a D-glucopyranosyl

(109) W. N. Haworth, E. L. Hirst and A. Learner, *J. Chem. Soc.*, 1040 (1927).
(110) J. C. Irvine, A. W. Fyfe and T. P. Hogg, *J. Chem. Soc.*, **107,** 524 (1915).
(111) J. C. Irvine and J. Patterson, *J. Chem. Soc.*, **121,** 2696 (1922).
(112) J. Böeseken and H. Couvert, *Rec. trav. chim.*, **40,** 354 (1921).
(113) M. Bergmann, *J. Chem. Soc.*, **123,** 1277 (1923).
(114) W. N. Haworth and W. H. Linnell, *J. Chem. Soc.*, **123,** 294 (1923).
(115) W. N. Haworth and J. G. Mitchell, *J. Chem. Soc.*, **123,** 301 (1923).
(116) E. H. Goodyear and W. N. Haworth, *J. Chem. Soc.*, 3136 (1927).
(117) Ref. 81, pp. 34–46.

D-fructofuranoside. The methylation results did not determine the two alpha or beta glycosidic configurations about the biose junction, and XV, like II, therefore had four possible configurations.



XV
Sucrose (Haworth-Hirst, 1927)

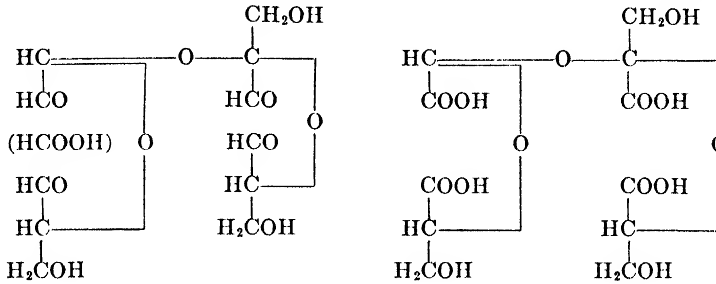
3. *Confirmatory Evidence*

Constitution XV for sucrose has up to the present satisfied all demands made upon it. Like its precursors, I and II (page 6), it was not incompatible with physical properties of sucrose such as the magnetic rotation,[118] or the parachor,[119] although the latter claim has been denied.[120] Von Lippmann[121] lists a great many early determinations of the physical properties of the sugar; more recent measurements include the heat of combustion,[122] the molecular weight in liquid ammonia,[123] and various optical and electrical constants.[124]

The ready condensation of sucrose with excess triphenylmethyl chloride in pyridine[125] to a tri-trityl ether is, however, more easily explained by XV or II, which have three, instead of two (*cf.* I), primary alcohol groups to react selectively in the condensation. Fleury and Courtois[126] oxidized sucrose for twenty-four hours at 14° with an excess of suitably buffered periodic acid and found that three moles of the oxidant were consumed and one mole of formic acid was eliminated. This highly selective oxidant is known to cleave unsubstituted 1,2 glycols quantitatively to two carbonyl groups and to eliminate the center carbon atom

(118) W. H. Perkin, *J. Chem. Soc.*, **81**, 177 (1902).
(119) S. K. Ray, *J. Indian Chem. Soc.*, **11**, 843 (1934).
(120) F. Hartley and W. H. Linnell, *Quart. J. Pharm. Pharmacol.*, **11**, 714 (1938).
(121) Ref. 2, pp. 596–683.
(122) W. Świetoslawski and Mlle. H. Starczewska, *Bull. soc. chim.*, **31**, 654 (1922).
(123) L. Schmidt and L. Haschek, *Monatsh.*, **59**, 328 (1932).
(124) E. L. Palumbo, *Ann. Physik*, **79**, 533 (1926).
(125) K. Josephson, *Ann.*, **472**, 230 (1929).
(126) P. Fleury and J. Courtois, *Compt. rend.*, **214**, 366 (1942); **216**, 65 (1943); *Bull. soc. chim.*, **10**, 245 (1943).

of an unsubstituted 1,2,3 triol as formic acid.[127] Formula XV for sucrose has the 1,2 and the 1,2,3 hydroxyl units necessary to explain the experimental results and the oxidized product would have the structure XVI. The absence of formaldehyde from the oxidation products was



XVI XVII

definite proof that the fifth position in both the D-glucose and D-fructose halves of XV was substituted. These interpretations were confirmed by oxidizing the tetraaldehyde XVI to the corresponding tetracarboxylic acid XVII, whose barium and strontium salts had the correct analyses. Complete acid hydrolysis of XVII then yielded two moles of D-glyceric acid, one mole of glyoxylic acid and one mole of glycolic aldehyde, the latter presumably arising by the decarboxylation of the mole of hydroxypyruvic acid expected from the upper half of the oxidized D-fructose unit. In similar fashion, the acid hydrolyzate of XVI, after high-pressure hydrogenation over Raney nickel at 140°, yielded a mixture of glycerol and ethylene glycol. Hockett and Zief[128] confirmed the above observations and showed that the ratio of glycerol to glycol was not far from the theoretical value of 82:18 by weight. The oxidation of sucrose with lead tetraacetate, instead of with periodate, proceeded with no appreciable cleavage of the glycosidic link and also yielded the tetraaldehyde XVI. These oxidations, together with the constitutional studies on XVI and XVII, obviously provide an independent and decisive proof of the size and position of the cyclic structures present in sucrose.

IV. Configuration of the Glycosidic Bonds in Sucrose

1. *Proof of the α-D-Glucopyranoside Configuration*

The researches of Hudson,[129] together with those of Dubrunfaut, Tanret, Lowry and others, which he reviewed, clearly established the

(127) E. L. Jackson, "Organic Reactions," J. Wiley and Sons, New York, Vol. 2, pp. 341–375 (1944). Periodate oxidations are systematically reviewed.

(128) R. C. Hockett and M. Zief, Abstracts of Papers, 110th Meeting, American Chemical Society, Atlantic City, New Jersey, p. 5R (1946).

(129) C. S. Hudson, *J. Am. Chem. Soc.*, **32**, 889 (1910).

fact that D-glucose existed in two distinct crystalline forms with specific rotations of $[\alpha]^{20}_D$ +113° and +19° in water. The more dextrorotatory isomer was arbitrarily defined as the alpha form, and the less dextrorotatory, as the beta form.[130] In aqueous solution both forms came into dynamic equilibrium with each other at a rate increased markedly by hydroxyl ions, but only slightly by hydrogen ions, so that the minimum rate of the mutarotation occurred in the pH range 3 to 4.[131] The attainment of this equilibrium was reflected accurately in the mutarotation of freshly prepared aqueous solutions of either α- or β-D-glucose to the final specific rotation of +52.5°, which was approached according to the first-order rate-of-change relationship calculated for balanced reactions.[132] Today the mechanism of this interconversion is recognized as a prototropic shift involving no overall change in the pyran cyclic structure.[133] Although only one crystalline form of D-fructose, β-D-fructopyranose with $[\alpha]^{20}_D$ −133.5° in water, is known, a very rapid mutarotation to a final value of $[\alpha]^{20}_D$ −92° shows that at least one other, less levorotatory isomer is capable of existence in solution.

Equipped with most of the above information, Hudson[134] was able to repeat and extend in a more precise way the experiments by O'Sullivan and Tompson[55] on the inversion of sucrose by invertase. The inversion was finally studied near 0° because the mutarotation of the liberated D-fructose was too swift to be conveniently followed at the temperature of 30° adopted for the preliminary work. The strong invertase solution was adjusted with acetic acid to the slightly acid range (pH 3 to 4) in which the rate of inversion was near its maximum and the rates of mutarotation of the liberated reducing sugars near their minima. Adopting a technique originated by Armstrong[57] in his study of the enzymatic hydrolysis of methyl α- and β-D-glucoside, massive amounts of the invertase preparation were suddenly added to an aqueous solution of sucrose, so that complete inversion was brought about within two or three minutes. Fig. 1 is reproduced from Hudson's article[134] and records the combined mutarotation of the liberated D-glucose and D-fructose through the initial period. The overall rate of mutarotation was faster in experiment II than in experiment I because approximately five times the amount of acetic acid, and, incidentally, of invertase, was present in the former case.

In the earlier work at 30°, a portion of the completely inverted,

(130) C. S. Hudson, *J. Am. Chem. Soc.*, **31**, 66 (1909).
(131) C. S. Hudson, *J. Am. Chem. Soc.*, **29**, 1571 (1907); **31**, 1136 (1909).
(132) C. S. Hudson, *Z. physik. Chem.*, **44**, 487 (1903).
(133) T. M. Lowry and I. J. Faulkner, *J. Chem. Soc.*, **127**, 2883 (1925).
(134) C. S. Hudson, *J. Am. Chem. Soc.*, **30**, 1564 (1908); **31**, 655 (1909).

mutarotated solution was used as a solvent in which to observe the mutarotation of crystalline α-D-glucose in exactly the circumstances of the inversion. The unimolecular rate constant found for the mutarotation proved to be identical with a value calculated from the later, nearly linear portion of the plot representing the optical changes connected with the inversion of the sucrose. This correspondence made it probable that

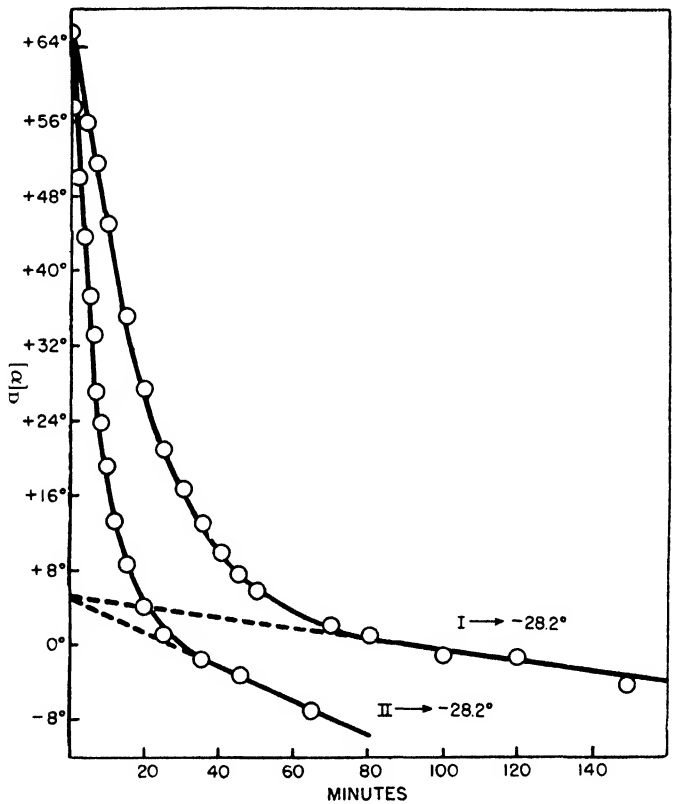


FIG. 1.[134]—Decrease with time in specific rotation at 0° of approximately 5% sucrose solutions completely and quickly inverted by invertase. Plot II corresponds to a somewhat higher acidity than plot I.

in Fig. 1 the mutarotation of D-glucose was solely responsible for the later, nearly linear portions of plots I and II. It followed that extrapolation of these portions to zero time gave the specific rotation corresponding to complete inversion of the sucrose, to no mutarotation of the liberated D-glucose and to complete mutarotation of the D-fructose to its known, final, specific levorotation of $[\alpha]^0_D$ −101°. The extrapolation in both experiments indicated a specific dextrorotation of +5°. This value,

together with the fact that 1 g. of sucrose yielded 0.525 g. each of D-glucose and of D-fructose, made it possible to calculate the specific rotation, $x°$, of the freshly liberated D-glucose by the method of mixtures:

$$(-101°)(0.525) + 0.525x° = 5° \times 1.0$$

The result, $[\alpha]^0\text{D}$ $+110°$ with an error of not more than $\pm 2°$, showed that prior to mutarotation the D-glucose was the ordinary α-form of rotation approximately $+109°$, now known as α-D-glucopyranose. Sucrose, then, was an α-D-glucoside. Inspection of Fig. 1 also shows that after inversion but before mutarotation the sum of the rotations contributed by the α-D-glucose and the D-fructose remained very close to the specific rotation of 66° possessed by the original sucrose. The relationship:

$$(109°)(0.525) + 0.525y° = 66° \times 1.0$$

gave $y°$, the specific rotation at 0° of the newly liberated D-fructose, as $+17°$.

The above reasoning implied that as a first approximation the initial rapid decrease in the specific rotation of the inverted sucrose solution to $+5°$ could be attributed to the mutarotation of the D-fructose, of $[\alpha]^0\text{D}$ $+17°$, to the final, normal equilibrium value of $-101°$. Unimolecular rate constants, calculated from the data of experiments I and II on this assumption, were indeed in approximate agreement with those found for pure crystalline D-fructose dissolved in the same completely inverted, completely mutarotated solutions, although the absolute values differed nearly threefold because of the increased acidity present in experiment II. The validity of the above implication was later upheld by Bailey and Hopkins,[135] who studied the inversion of sucrose with varying amounts of strong, maltase-free invertase solution at 17° and in the pH range 4.6 to 6.1. The refined mathematical treatment to which they subjected their polarimetric data took account of the slow mutarotation of the α-D-glucose during the initial period and revealed the specific rotation of the mutarotating D-fructose at any time. Extrapolation of these specific rotations to zero time gave $[\alpha]^{17}\text{D}$ $+17°$ to $+15°$ in all cases. Hudson's observation that the inversion of sucrose yielded D-glucose and D-fructose without causing appreciable alteration in the initial observed rotation was also carefully checked. On another occasion,[136] similar experiments were carried out at 20° with solutions heavily buffered to pH 4.5, and the data were later used by Isbell and Pigman[137] to show that the

(135) K. Bailey and R. H. Hopkins, *Biochem. J.*, **27**, 1957 (1933).

(136) C. B. Purves and C. S. Hudson, *J. Am. Chem. Soc.*, **56**, 702 (1934).

(137) H. S. Isbell and W. W. Pigman, *J. Research Natl. Bur. Standards*, **20**, 773 (1938).

rate constant for the mutarotation of the liberated α-D-glucose was in excellent agreement with the value found for the pure crystalline sugar dissolved in acetate buffer at the same *p*H and temperature. After a simplified mathematical treatment had made allowance for the initial mutarotation of the D-glucose, the calculated rate constant for the D-fructose moiety was in close agreement with that observed for the crystalline sugar in the same circumstances. Since the mutarotation of the D-fructose liberated from sucrose was then known to involve a furanose to pyranose ring shift, the concordance was evidence that the reverse change occurred when crystalline β-D-fructopyranose was dissolved in water. The activation energy of this ring shift was 14,900 calories per mole, whereas a value of about 17,000 calories was characteristic of mutarotations of a pure pyranose (glucose) type. The latter occurred 9,[137] 11[134] or 27[135] times more slowly, depending upon the experimental conditions. Further calculation from the results of Isbell and Pigman also confirms the specific dextrorotation of +17° found by Hudson and by Bailey and Hopkins for D-fructose newly liberated during the inversion of sucrose.

2. *Proof of the β-D-Fructofuranoside Configuration*

In 1909, it was logical to call this unisolated D-fructose, the alpha, and the known crystalline levorotatory variety, the beta isomer, although other considerations made it necessary to regard the magnitude of the dextrorotation, +17°, as "abnormal."[134] The later recognition that the two isomers differed in ring structure accounted for the "abnormality" in rotation and at the same time demolished the basis for assigning either an alpha or a beta configuration to the dextrorotatory D-fructofuranose. The proper configuration for the D-fructofuranoside moiety of sucrose was eventually found by a series of researches on the methyl D-fructofuranosides which developed from the work of Menzies,[103] who reviewed the earlier literature. Menzies condensed pure D-fructose at room temperature with dry methanol containing a little hydrogen chloride, care being taken to arrest the reaction at the end of the initial swift decrease in levorotation, and to use methanol free from traces of acetone. The latter was known to condense preferentially with the sugar and the resulting isopropylidene derivatives had grossly contaminated earlier preparations of the methyl D-fructosides.[104] Successive extractions of the crude product with ethyl acetate yielded a series of sirups with specific dextrorotations ranging from 35° to 9° and with good carbon, hydrogen and methoxyl analyses for methyl fructoside. As already mentioned, complete methylation and subsequent hydrolysis of this "gamma" methyl D-fructoside sirup to 1,3,4,6-tetramethyl-D-fructose showed that

it possessed the furanose structure.[103] The sirup therefore consisted of the methyl α- and β-D-glycosides in variable relative amounts. Schlubach and Rauchalles[138] then found that a purified invertase solution hydrolyzed about one-third of a similar methyl D-fructofuranoside sirup to D-fructose and that the remaining two-thirds was unaffected by the enzyme. When a mathematical slip is corrected, the resulting increase in the copper-reducing power and in the levorotation of their solution corresponded to specific rotations of $[\alpha]^{20}_{D}$ +54.3° for the constituent stable to the enzyme and of −51.5° for that which was hydrolyzed. Applying the usual convention for naming α- and β-glycosides in the D series,[130] the latter D-fructoside was the beta-isomer since it was the more levorotatory. The authors were aware that methyl glycosides usually have optical rotations substantially greater in absolute magnitude than those of the corresponding forms of the reducing sugar, and that methyl β-D-fructofuranoside, like Armstrong's methyl β-D-glucoside,[57] should give a much more dextrorotatory β-D-fructofuranose as the initial product of hydrolysis. The truth of this inference was established by Purves and Hudson,[136] who employed relatively large amounts of maltase- and β-D-glucosidase-free invertase at 20° and *p*H 4.5 to hydrolyze the unstable constituent of the methyl D-fructoside sirup at a rate comparable to that of the mutarotation of the liberated D-fructose.

An approximately 5% solution of the sirup, when observed on a saccharimeter in a 4-dm. tube, had an initial rotation of +0.73° Ventzke increasing to +3.71°V after five to six minutes and then decreasing to the final value of −7.9°V in about two hours. Readings such as these proved that the magnitude of the initial increase in dextrorotation was far outside the observational error. Fig. 2, plot I, records the corresponding specific rotations as calculated from the initial concentration of the whole methyl D-fructoside sirup. From the optical rotations and copper-reducing values, it was shown that the initial observed rotation of +0.73°V was the algebraic sum of +14.18°V, contributed by unhydrolyzed components, and of −13.45°V, contributed by the methyl β-D-fructofuranoside, whose concentration in terms of D-fructose was 2.13%. The latter contribution decreased to zero as hydrolysis proceeded, and its magnitude at any time may be readily calculated from a plot of the percentage hydrolysis as determined from the observed increase in reduction. The difference between this value and the observed rotation at the corresponding time yields the rotation contributed by the liberated D-fructose, increased by the unchanged quantity +14.18°V. The specific mutarotation of the D-fructose, given in plot III, clearly corresponds to that of an initial form much more dextrorotatory than $[\alpha]_{D}$ −51°, the rotation

(138) H. H. Schlubach and Gertrud Rauchalles, *Ber.*, **58**, 1842 (1925).

found for the parent methyl glycoside. Parallel experiments on the partial hydrolysis by invertase of a benzyl D-fructofuranoside mixture[139] also exhibited an increase in the observed dextrorotation from the initial value of +1.98°V to +2.76°V before the subsequent decrease to constancy at +0.89°V commenced. The corresponding changes in the specific rotation of the mixture (plot II) indicated that the glycoside

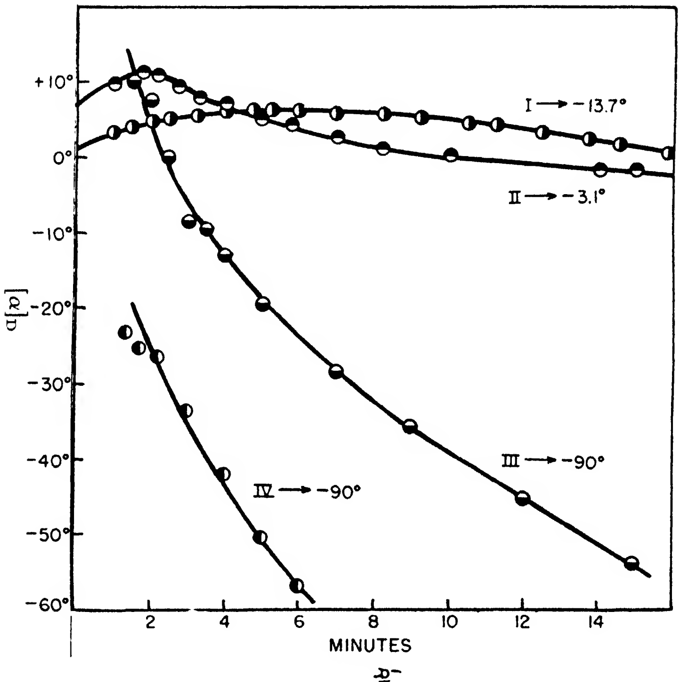


FIG. 2.—Partial hydrolysis of fructofuranoside sirups with invertase. Plot I, change in specific rotation of the entire methyl fructoside sirup; plot II, same for benzyl fructoside sirup; plot III, change in calculated specific rotation of fructose from methyl fructoside; plot IV, same for fructose liberated from benzyl fructoside sirup.

unstable to invertase was of the beta configuration. This indication is strengthened by the specific rotations calculated for the liberated D-fructose (plot IV), which strongly suggest that the initial form was more dextrorotatory than the value of $[\alpha]^{20}_D$ −27.5° calculated for the corresponding benzyl β-D-fructofuranoside.

Comparisons made with the same invertase solutions showed that the methyl and benzyl β-D-fructofuranosides were hydrolyzed 13.5[136] and 5.1[139] times, respectively, more slowly than sucrose, and that the rates

(139) C. B. Purves and C. S. Hudson, *J. Am. Chem. Soc.*, **59**, 49 (1937).

could not be regarded as nearly instantaneous. The actual times required in the experimental conditions were of the order of thirty and ten minutes. During these periods, the observed optical changes reflected not only the mutarotation of the liberated D-fructose but also the rate at which the concentration of the latter increased as hydrolysis proceeded. Plots III and IV of Fig. 2 therefore have a complex shape and this consideration, plus the very large accumulated error in the calculation of the initial rotations, renders an extrapolation to zero time uncertain. The plots neither confirm nor deny the specific rotation of +17° found for β-D-fructofuranose by the previous studies on the practically instantaneous inversion of sucrose. Neither the methyl nor the benzyl β-D-glycosides have as yet been isolated in a pure crystalline form.

When the original methyl D-fructofuranoside sirup was fermented with yeast, the unstable beta isomer was selectively eliminated and the residue yielded a crystalline methyl D-fructoside melting at 81°[139] and with $[\alpha]^{20}{}_D$ +93° in water.[140] The ring structure of this new isomer was proved to be furan by methylation to the liquid tetramethyl derivative, of $[\alpha]^{20}{}_D$ +129.4°, and subsequent hydrolysis to 1,3,4,6-tetramethyl-D-fructofuranose (structure IX) with the correct specific rotation of +29.8° in water.[139] Both the methyl D-fructoside and its fully methylated derivative were therefore of the alpha configuration, since the latter was more dextrorotatory than the tetramethyl-D-fructose and also since the former was more dextrorotatory than the isomer, of $[\alpha]^{20}{}_D$ −51°, unstable to invertase. Similar work with the benzyl D-fructofuranoside sirup[139] produced the crystalline alpha isomer, melting point 89°, $[\alpha]^{20}{}_D$ +45.7° in water, the liquid tetramethyl derivative, $[\alpha]^{20}{}_D$ +83.3° in chloroform and, after acid hydrolysis of the latter, 1,3,4,6-tetramethyl-D-fructofuranose.

Although dilute aqueous acid hydrolyzed the methyl and benzyl α-D-fructofuranosides approximately 8[64,141] and 16[64,139] times as rapidly as sucrose, their method of preparation showed them to be unaffected by any of the enzymes active in a fermenting yeast suspension. Purified yeast invertase, proven free of α-D-glucosidases (maltases), could therefore contain no enzyme capable of hydrolyzing the above two α-D-fructofuranosides, but did contain constituents that readily cleaved the beta isomers and also sucrose. The latter is accordingly a β-D-fructofuranoside. When the evidence is put in this way, the present uncertainties as to whether purified invertase preparations include one or a number of β-D-fructofuranosidases,[142,143] and whether or not sucrose, methyl and

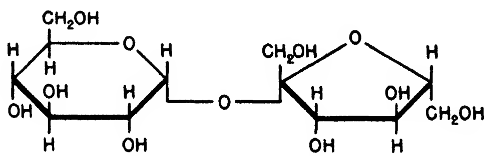
(140) C. B. Purves and C. S. Hudson, *J. Am. Chem. Soc.*, **56**, 708 (1934).

(141) C. B. Purves, *J. Am. Chem. Soc.*, **56**, 1969 (1934).

(142) J. M. Nelson, *Chem. Revs.*, **12**, 1 (1933). A review of the literature.

(143) Mildred Adams, N. K. Richtmyer and C. S. Hudson, *J. Am. Chem. Soc.*, **65**, 1369 (1943).

benzyl β-D-fructofuranosides are hydrolyzed by the same member of this enzyme class, seem to be irrelevant. The argument is likewise unaffected by the existence of certain "abnormal" invertases that fail to invert sucrose according to first-order kinetics,[142,143] or by the fact that sucrose, as an α-D-glucoside, is also hydrolyzed by α-D-glucosidases. Pottevin[144] was the first to demonstrate that sucrose could be inverted, and crude methyl-D-fructoside partly hydrolyzed, by invertase preparations that were devoid of enzymes of the maltase type. Numerous later workers have upheld his clear differentiation between the maltases and the invertases. The conclusion that sucrose is α-D-glucopyranosyl-β-D-fructofuranoside is expressed in the structure XVIII, which is a perspective formula of the type introduced by Haworth.[145]



XVIII
Sucrose (α-D-glucopyranosyl-β-D-fructofuranoside)

3. *Other Relevant Observations*

The structure indicated for sucrose (XVIII) is in agreement with other lines of evidence that are available. When a 0.9% solution of the sugar in dry methanol containing 0.1% of hydrogen chloride was kept at 20°, cleavage into D-glucose and methyl D-fructofuranosides was complete in about thirty-four minutes.[146] The nonreducing portion of the product, when analyzed by means of invertase, contained 54 to 60% of methyl β-D-fructofuranoside with the correct specific rotation of $-51 \pm 1°$. When the methanolysis was allowed to proceed for an hour, the proportion of the beta isomer had decreased to 50%, which was the value obtained when the pure methyl α-D-fructofuranoside was brought into equilibrium with the beta isomer by acid methanol used under the same conditions.[141] Although the observations were consistent with interpretation (*a*) of the accompanying diagram, they did not exclude the possibility (*b*) that the initial cleavage of the disaccharide yielded D-glucose and some form of D-fructose. The latter was known to yield the equilibrium mixture of methyl furanosides with comparable rapidity

(144) H. Pottevin, *Compt. rend.*, **136**, 169 (1903).
(145) Ref. 81, pp. 90–96.
(146) C. B. Purves and C. S. Hudson, *J. Am. Chem. Soc.*, **56**, 1973 (1934).

under the experimental conditions, but the possibility of an initial preferred formation of the beta isomer was not considered.[136] Berner[147]

(a)

$$\begin{array}{ccccc} \text{Sucrose} & \xrightarrow[\text{HCl (fast)}]{\text{MeOH}} & \alpha\text{-D-Glucose} & + & \text{Methyl } \beta\text{-D-fructofuranoside} \\ & & \downarrow \text{(slow)} & & \uparrow\downarrow \text{(fast)} \\ & & & & \text{Alpha isomer} \\ & & & & \downarrow \text{(slow)} \\ & & \text{Methyl D-glucosides} & & \text{Methyl D-fructopyranosides} \end{array}$$

(b)

$$\begin{array}{ccccc} \text{Sucrose} & \xrightarrow[\text{HCl (fast)}]{\text{MeOH}} & \alpha\text{-D-Glucose} & + & \text{D-Fructofuranose(?)} \\ & & & & \downarrow \text{(fast)} \\ & & & & \text{Methyl } \beta\text{-D-fructofuranoside} \\ & & & & \uparrow\downarrow \text{(fast)} \\ & & & & \text{Alpha isomer} \end{array}$$

also cleaved sucrose to D-glucose and a methyl D-fructofuranoside mixture by heating a methyl alcoholic solution of the disaccharide at 150–155° for eight to ten hours in an atmosphere of nitrogen. Since extractions of the methyl D-fructoside with ethyl acetate gave fractions with specific dextrorotations as high as +54°, Berner inferred that the initial product of the sucrose cleavage was methyl α-D-fructofuranoside and that the same configuration was present in sucrose itself. This erroneous inference, however, was drawn in ignorance of the facts that methyl α-D-fructofuranoside has a specific rotation of +93°, that its equilibrium mixture with the beta isomer has $[\alpha]_D$ about +21° and that extraction of the mixture by solvents like ethyl acetate tends to concentrate the alpha isomer in the extract.[140]

Another research of a somewhat similar nature commenced with the D-fructose 1,6-diphosphate[148] isolated from yeast fermentations. Morgan[149] treated this substance with methanol containing 0.5% hydrogen chloride for twenty-four hours and obtained the corresponding methyl glycosides, which were isolated in two fractions as amorphous barium salts with specific rotations of +19.7° and −10.4°. Although each salt was shown to be predominantly of a furan type,[148] the loss of 10 to 13% of the phosphoric acid during the condensation, together with the rather drastic nature of the latter, suggests that the salts might have contained strongly levorotatory D-fructopyranoside derivatives as impurities. For this reason little theoretical significance can be attached to the difference between the specific rotations of the two salts, to the fact that invertase hydrolyzed a portion of the dextrorotatory, rather than the levorotatory, fraction, or to the inference that the enzyme acted upon α- rather than

(147) E. Berner, *Ber.*, **66**, 1076 (1933).
(148) W. T. J. Morgan and R. Robinson, *Biochem. J.*, **22**, 1270 (1928).
(149) W. T. J. Morgan, *Biochem. J.*, **21**, 675 (1927).

β-D-fructosides. The opinion that the work argued for the presence of an α-D-fructofuranoside unit in sucrose[150] also appears to be ill-founded.

The conclusion that sucrose is an α-D-glucopyranoside is consistent with the recovery of its octaacetate, unchanged and in 96% yield, after being heated in chloroform solution with titanium tetrachloride.[151] This reagent is known to produce little change in simple, fully acetylated α-D-glucopyranosides,[152] but to cause the extensive isomerization of the beta to the alpha isomers.[153]

V. The Related Structure of Isosucrose

Butlerow's observation[154] that an optically inactive sugar-like substance was produced when tri(oxymethylene) was boiled with milk of lime led to the preparation by Loew[155] of a sirup named formose, which was thought to be a hexose. The action of alkali on glyceraldehyde, or acrolein dibromide, produced a similar sirup, α-acrose,[156] from which D,L-glucose phenylosazone in pure condition was obtained.[157] The subsequent generation of D,L-fructose therefrom, together with the interconversion of D-fructose and D-glucose, reduced the problem of an ultimate sucrose synthesis to a proper condensation of the two hexose units. It seems reasonable to discount claims that the disaccharide was produced directly from mixtures such as formaldehyde and alkali[158] or from carbon dioxide, acetylene and water gas,[159] even when the aid of magnetic, electrical or luminescent influences was invoked. Early synthetical attempts based on the chemical combination of D-fructose and D-glucose were doubtless numerous but also unsuccessful, since none were found recorded in the literature.

By 1920, it was recognized that a successful chemical synthesis would probably require the use of a D-fructose maintained in the proper cyclic structure by the presence of substituents. The substituent groups would have to be stable enough to survive the conditions required in condensation with a suitable D-glucose derivative, but at the same time be capable of ready removal by agents that failed to affect any sucrose

(150) H. Pringsheim, "The Chemistry of the Monosaccharides and of the Polysaccharides," McGraw-Hill and Co., New York, p. 99 (1932).
(151) C. D. Hurd and S. M. Cantor, *J. Am. Chem. Soc.*, **60**, 2677 (1938).
(152) E. V. Piel and C. B. Purves, *J. Am. Chem. Soc.*, **61**, 2978 (1939).
(153) E. Pacsu, *J. Am. Chem. Soc.*, **52**, 2563, 2568, 2571 (1930).
(154) A. Butlerow, *Ann.*, **120**, 295 (1861); *Compt. rend.*, **53**, 145 (1861).
(155) O. Loew, *Ber.*, **22**, 470, 478 (1889). A review of the literature is included.
(156) E. Fischer and J. Tafel, *Ber.*, **20**, 3384 (1887); **22**, 97 (1889).
(157) E. Fischer, *Ber.*, **23**, 370 (1890).
(158) C. A. Stewart, *French Pat.*, 381,292 (1907); *Chem. Abstracts*, **3**, 1104 (1909).
(159) L. H. Roman, *Brit. Pat.*, 374,044 (1932); *Chem. Abstracts*, **27**, 4123 (1933).

formed. These considerations led Irvine and his collaborators[160,161] to cleave a fully acetylated ethyl D-fructofuranoside mixture, or triacetylinulin[162,163] with acetyl chloride or bromide to the corresponding, uncrystallized tetraacetyl-D-fructofuranosyl halide. This reaction was later shown to depend on the adventitious presence of traces of the hydrogen halide.[164] Water converted the tetraacetyl-D-fructofuranosyl chloride to the corresponding, uncrystallized tetraacetyl-D-fructose, of specific rotation +31.5 to +38.7° in water, whose furan structure was proved by the methylation method.[161] An equimolecular mixture of this tetraacetyl-D-fructose and 2,3,4,6-tetraacetyl-D-glucopyranose[165] (the mixture sometimes being prepared by the direct cleavage of sucrose octaacetate by acetyl halide[162]) was shaken for many hours in benzene or chloroform solution containing phosphoric anhydride as a dehydrating agent. In some experiments anhydrous zinc chloride was also added. After the condensation, extraction of the crude product with water removed unchanged starting materials before the search for sucrose octaacetate in the extracted residue was undertaken. This search was invariably unsuccessful, not only for Irvine and his collaborators,[160–163] but also for Zemplén and Gerecs,[166] Georg[167] and Binkley and Wolfrom,[164] although the latter workers used highly sensitive chromatographic methods in their investigation. The condensation of 2,3,4,6-tetraacetyl-β-D-glucopyranose with tetraacetyl-D-fructofuranosyl chloride,[161] or with the corresponding crude tetrabenzoyl-D-fructosyl bromide[168] also failed to yield the corresponding sucrose derivative. A claim that sucrose octaacetate was formed when the two tetraacetates were condensed in the presence of anhydrous zinc chloride[169] could not be substantiated[160–161,166–168] and was later withdrawn.[170]

Although the above condensations definitely failed to yield sucrose octaacetate, Irvine and his collaborators showed that they did produce, in addition to uncrystallized products, some D-glucose pentaacetate, some

(160) J. C. Irvine, J. W. H. Oldham and A. F. Skinner, *J. Soc. Chem. Ind.* (London), **47,** 494 (1928).

(161) J. C. Irvine, J. W. H. Oldham and A. F. Skinner, *J. Am. Chem. Soc.*, **51,** 1279 (1929).

(162) J. C. Irvine and J. W. H. Oldham, *J. Am. Chem. Soc.*, **51,** 3609 (1929).

(163) J. C. Irvine and E. T. Stiller, *J. Am. Chem. Soc.*, **54,** 1079 (1932).

(164) W. W. Binkley and M. L. Wolfrom, *J. Am. Chem. Soc.*, **68,** 2171 (1946).

(165) E. Fischer and K. Delbrück, *Ber.*, **42,** 2776 (1909).

(166) G. Zemplén and A. Gerecs, *Ber.*, **62,** 984 (1929).

(167) A. Georg, *Helv. Chim. Acta*, **16,** 130 (1933).

(168) F. Klages and R. Niemann, *Ann.*, **529,** 185 (1937).

(169) A. Pictet and H. Vogel, *Compt. rend.*, **186,** 724 (1928); *Helv. Chim. Acta*, **11,** 436 (1928); *Ber.*, **62,** 1418 (1929).

(170) A. Pictet, *Helv. Chim. Acta*, **16,** 144 (1933).

isotrehalose (β-D-glucopyranosyl-β-D-glucopyranoside) octaacetate and not more than 5% of a new disaccharide acetate named isosucrose octaacetate.[160,161] The latter melted at 131–132°, had a specific rotation $[\alpha]_D$ +19.9° in chloroform, and, after deacetylation, gave isosucrose, melting at 179° and with a specific rotation of +34.2° in water. These observations were confirmed by later workers;[164,166–168] "Saccharose D" of Pictet and Vogel[169] was eventually shown to be an impure sample of isosucrose,[171] and the same verdict probably also applies to the reducing substance, of specific rotation +34.8° and melting over the wide range of 152 to 172°, recently prepared by Schlubach and Middelhoff.[172] Their claim that isosucrose was a reducing glucosylfructose is quite inconsistent with the nonreducing character of the disaccharide noted by other workers, and also with the results obtained when isosucrose was methylated with dimethyl sulfate and alkali. The resulting octamethylisosucrose[173] was prepared in 60 to 70% overall yield as a liquid, which was hydrolyzed by 0.01 *N* hydrochloric acid even more readily than octamethylsucrose to the same equimolecular mixture of 2,3,4,6-tetramethyl-D-glucopyranose and 1,3,4,6-tetramethyl-D-fructofuranose. In similar fashion, isosucrose itself underwent acid hydrolysis to invert sugar, with the correct specific levorotation of −20°,[161] more readily than sucrose. These observations proved that isosucrose was one of the four D-glucopyranosyl D-fructofuranosides depicted in structure XV and differed from sucrose only in the configuration about the glycosidic bonds.

The probable configuration of isosucrose was systematically discussed by Georg,[171] who thought that the yield was much greater when the beta anomer, rather than the equilibrium mixture or the alpha anomer, of 2,3,4,6-tetraacetyl-D-glucose was used in the condensation. Analogy with tetraacetyl-D-fructopyranosyl chloride,[174] which yielded the acetylated methyl α-D-fructo-pyranoside[175] instead of the beta isomer produced by most acetohalogen sugars, when condensed with methanol, suggested to Georg that tetraacetyl-D-fructofuranosyl chloride would most probably produce α-D-fructofuranosides when similarly condensed. On this reasoning isosucrose was probably β-D-glucopyranosyl α-D-fructofuranoside (XIX). Klages and Niemann,[168] in control experiments, condensed 2,3,4,6-tetraacetyl-D-glucopyranose with itself in the presence of phosphoric anhydride and came to the conclusion that 88% reacted in the beta configuration, regardless of whether the pure α- or β-tetraacetate was

(171) A. Georg, *Helv. Chim. Acta*, **17**, 1566 (1934).

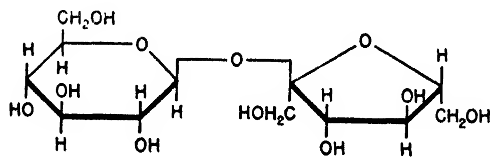
(172) H. H. Schlubach and B. Middelhoff, *Ann.*, **550**, 134 (1942).

(173) J. C. Irvine and D. Routledge, *J. Am. Chem. Soc.*, **57**, 1411 (1935); *Nature*, **134**, 143 (1934).

(174) H. H. Schlubach and G. A. Schröter, *Ber.*, **61**, 1216 (1928).

(175) E. Pacsu and F. B. Cramer, *J. Am. Chem. Soc.*, **57**, 1944 (1935).

used for the condensation. When alkylated with ethyl bromide in the presence of silver carbonate, either anomer yielded the same 75:25 mixture of ethyl tetraacetyl-α- and β-D-glucopyranosides. The reverse occurred with 1,3,4,6-tetraacetyl-D-fructofuranose from inulin, and dextrorotatory, presumably alpha, forms predominated both in the condensation with phosphoric anhydride and with ethyl bromide. The fact that the corresponding D-fructofuranosyl chloride also yielded dextrorotatory, presumably alpha, glycosides when shaken with alcohol and silver carbonate made the authors remark that it was impossible to prepare β-D-fructofuranosides by this route.



XIX
Isosucrose (β-D-glucopyranosyl-α-D-fructofuranoside)

The above arguments in support of structure are weak because the condensations of 2,3,4,6-tetraacetyl-D-glucose gave mixtures of alpha and beta forms and the yields of isosucrose octaacetate were always small. Moreover, Pacsu[176] found that the crystalline tetraacetyl-D-fructopyranosyl chloride yielded 64 to 80% of methyl orthoesters, as well as methyl tetraacetyl-α-D-fructopyranoside (16 to 34%) when condensed with methanol. Unpublished work by Dr. C. S. Hudson and one of the present authors (C.B.P.) showed that the sirupy furanosyl chloride behaved in the same way and that speculations about the structure of the dextrorotatory glycosidic sirups prepared from it were complicated by the presence of 30 to 70% of methyl orthoesters. In these circumstances, the main support for structure XIX seems to depend on the observation that isosucrose was unaffected, not only by purified maltase and invertase[143,171] preparations, but also by all the enzymes in fermenting yeast. Isosucrose was therefore likely to be neither an α-D-glucopyranoside nor a β-D-fructofuranoside. Georg[171] noted, however, that the disaccharide was readily hydrolyzed by β-D-glucosidase from *Aspergillus niger*, although ordinary emulsin was not effective. The latter anomaly was considered to be unimportant, since other cases were known in which related enzyme preparations behaved differently toward the same substrate, for example, the variable action of different maltases toward

(176) E. Pacsu, *J. Am. Chem. Soc.*, **57**, 745 (1935); *Advances in Carbohydrate Chemistry*, **1**, 90 (1945).

trehalose (α-D-glucopyranosyl-α-D-glucopyranoside). Suggestions that isosucrose was probably a β-D-glucosyl β-D-fructoside[161,163,169] led to the inference that sucrose had either the β-α- or the α-α-D-glycosidic configuration,[161] but these early opinions have now entirely lost their original plausibility.

VI. Biochemical Syntheses in the Sucrose Series

The synthesis, by Bourquelot and Bridel[177] in 1912, of ethyl β-D-glucopyranoside by the reversible action of emulsin upon D-glucose in 85% aqueous alcohol, was followed by similar syntheses by the same authors of a whole series of α- and β-D-glucosides and D-galactosides, α-D-mannosides and α-L-arabinosides, the appropriate enzymes, reducing sugars and alcohols being used in each case. These successes naturally induced Bourquelot and Bridel to attempt a biochemical synthesis of sucrose from D-glucose, D-fructose and invertase,[178,179] but no sucrose was obtained, although various experimental conditions were used. Earlier synthetic attempts with invertase[179,180] had likewise led to no positive result.

It had been known from at least the time of Pasteur[26] that the presence of sodium or potassium phosphate aided the progress of a yeast fermentation.[180] Later intensive study showed that a complex group of enzymes (phosphatases and phosphorylases) was responsible for the phosphorylation, dephosphorylation and interconversion of D-glucose 6-phosphate, D-fructose 6-phosphate, D-fructose 1,6-diphosphate and similar substances in various types of cells and muscle tissue. Detailed reviews of the field are available.[181,182] A further advance was made in 1936, when Cori and Cori[183] noted that in certain circumstances well-washed frog muscle immersed in a sodium phosphate buffer utilized the inorganic phosphate to produce a new hexose phosphate (the Cori ester). This compound was later shown to be α-D-glucopyranose-1-phosphate[184,185] and yielded crystalline dipotassium and brucine salts. The Cori ester arose because

(177) E. Bourquelot and M. Bridel, *Compt. rend.*, **154,** 1737 (1912).

(178) E. Bourquelot and M. Bridel, *J. pharm. chim.*, [7] **9,** 321 (1914); *Chem. Abstracts*, **8,** 2553 (1914). See also O. A. Leonard, *Am. J. Bot.*, **25,** 78 (1938).

(179) A. W. Visser, *Z. physik. Chem.*, **52,** 257 (1905).

(180) A. Wroblewski, *J. prakt. Chem.*, **64,** 1 (1901).

(181) C. F. Cori, *Endocrinology*, **26,** 285 (1940).

(182) C. S. Hanes, *Proc. Roy. Soc. (London)*, **B128,** 421; **B129,** 174 (1940).

(183) C. F. Cori and Gerty T. Cori, *Proc. Soc. Exptl. Biol. Med.*, **34,** 702 (1936).

(184) C. F. Cori, S. P. Colowick and Gerty T. Cori, *J. Biol. Chem.*, **121,** 465 (1937).

(185) M. L. Wolfrom and D. E. Pletcher, *J. Am. Chem. Soc.*, **63,** 1050 (1941). References to the Cori ester are included. M. L. Wolfrom, C. S. Smith, D. E. Pletcher and A. E. Brown, *ibid.*, **64,** 23 (1942).

an enzyme, phosphorylase, promoted the equilibrium

$$\text{muscle glycogen} + H_3PO_4 \rightleftarrows \text{Cori ester}$$

and the ester accumulated in the system because the enzyme preparation used was fairly free of agents that caused its decomposition. This observation made it possible to prepare the Cori ester in quantity from glycogen, inorganic phosphate and muscle[184,186] or yeast[186] phosphorylases; or from starch, inorganic phosphate and phosphorylases derived from the juice of the potato and higher plants.[182] A convenient method of purification by adsorption on, and subsequent elution from, ion exchange resins was described by McCready and Hassid.[187] Conversely, the existence of the above equilibrium led to the synthesis of glycogen[183,186] and of starch[182] from the Cori ester, success in the syntheses naturally depending on the use of phosphorylase preparations reasonably free from interfering phosphatases and from diastase.

The idea that an enzyme of the phosphatase class, rather than an invertase, might be responsible for the natural synthesis of sucrose was not entirely new, but previous attempts to accomplish a synthesis of the crystalline sugar *in vitro* by this route had probably all failed.[188] Some microorganisms, however, were reported to metabolize disaccharides such as sucrose or trehalose much more readily than the constituent hexoses, and one of these organisms, *Pseudomonas saccharophilia* Doudoroff, was studied by Doudoroff and his colleagues.[188] Provided that this bacterium was grown on a medium containing sucrose, rather than D-glucose or D-fructose, the washed, carefully dried product caused the rapid removal of inorganic phosphate from a sucrose solution kept at pH 6.3 to 7.0 with a phosphate buffer. No removal of phosphate occurred when the sucrose was replaced by D-glucose, D-fructose or invert sugar, but in its presence some of the phosphate was recovered as the crystalline dipotassium salt of the Cori ester. D-Fructose was also liberated from the sucrose. These results were similar to those obtained by Kagan, Lyatker and Tsvasman[189] who used *Leuconostoc mesenterioides.* After Doudoroff[190] found a way of processing his bacterial extract to remove almost all interfering phosphatases and also invertase, he was able to demonstrate that the remaining phosphorylase probably promoted

(186) W. Kiessling, *Biochem. Z.*, **298,** 421 (1938); *Naturwissenschaften*, **27,** 129 (1939).

(187) R. M. McCready and W. Z. Hassid, *J. Am. Chem. Soc.*, **66,** 560 (1944).

(188) M. Doudoroff, N. Kaplan and W. Z. Hassid, *J. Biol. Chem.*, **148,** 67 (1943). Earlier references are cited.

(189) B. O. Kagan, S. N. Lyatker and E. M. Tsvasman, *Biokhimiya*, **7,** 93 (1942); *Chem. Abstracts*, **37,** 4760 (1943).

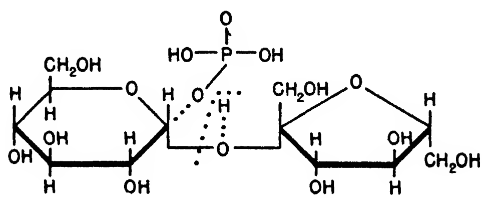
(190) M. Doudoroff, *J. Biol. Chem.*, **151,** 351 (1943).

the equilibrium

Cori ester + D-fructose $\rightleftarrows$ sucrose + inorganic phosphate,

the equilibrium constant being about 0.05 at pH 6.6 and 30°, and about 0.09 at pH 5.8.

The suggestion was that the components on either side of the equilibrium united, without the intervention of water, to form the sucrose–phosphoric acid adduct XX, which decomposed in either direction as shown by the dotted lines.

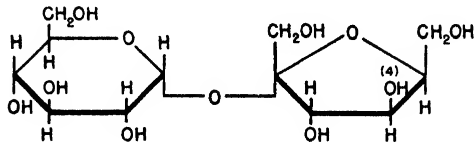


XX
Hypothetical sucrose–phosphoric acid complex

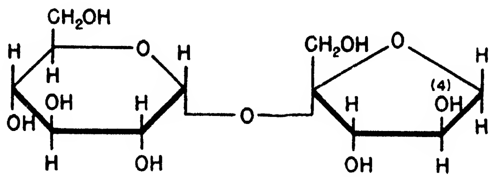
Hassid, Doudoroff and Barker[15] then mixed an invertase-free phosphorylase extract prepared from 6 g. of dry *Pseudomonas saccharophilia* Doudoroff bacilli with a barium acetate solution containing 15 g. of the dipotassium salt of the Cori ester and an equal weight of D-fructose. After incubation for twenty-four hours at pH 6.85 and 35° to 29°, analysis showed that about 3 g. of sucrose had been formed. The enzymes were then inactivated by a brief heating of the solution (300 cc.) at 80° and, after adjustment of the pH to 7.8, the precipitation of barium phosphates was completed by the addition of alcohol. Ion exchange resins were employed to withdraw the remaining electrolytes, including unchanged Cori ester, from the alcohol-free mother liquor, and a selective fermentation with *Torula monosa* removed the remaining D-fructose. After recovery, the residual sirup soon yielded crystalline sucrose, whose identity with an authentic sample was rigorously and unquestionably proved by a variety of physical and chemical methods. The substitution of the D-fructose used in the synthesis by the configurationally related L-sorbose[191] led to the preparation of a new nonreducing disaccharide melting at 178–180° and with a specific rotation of +33° in water, to which the structure XXI was assigned. In similar fashion, the use of a sirup consisting mainly of 2-ketoxylose (D-threopentulose) yielded the

(191) W. Z. Hassid, M. Doudoroff, H. A. Barker and W. H. Dore, *J. Am. Chem. Soc.*, **67**, 1394 (1945).

new nonreducing disaccharide XXII melting at 156–157° and with a rotation of +43° in water.[192]



XXI
α-D-Glucopyranosyl-α-L-sorbofuranoside



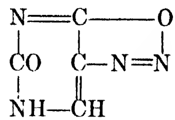
XXII
α-D-Glucopyranosyl-β-D-ketoxylofuranoside

Consideration of structures XXI and XXII showed that in each case oxidation with sodium periodate should liberate one mole of formic acid from the left-hand (D-glucopyranose) ring with the consumption of two moles of the oxidant, and that the right-hand (furan) structure should consume an additional mole. These expectations were fully borne out by experiment and the results in themselves sufficed to identify the ring structures of the two new disaccharides with those present in sucrose (XVIII). Both starch and glycogen are based upon α-D-glucopyranose units and were therefore synthesized without change in configuration from the Cori ester (α-D-glucopyranose 1-phosphate). This consideration strongly supported the conclusion that sucrose and the above disaccharides were also α-D-glucopyranosides, since all three were synthesized from the same substrate by similar phosphorylases. The specific nature of enzyme action also strongly indicated that the configurations of the right-hand portions of structures XXI and XXII probably differed from the D-fructose half of sucrose (XVIII) only in the orientation and nature of the addenda at the fourth position of the furan ring. Since sorbose is an L sugar, and since α-L-sorbose is configurationally more closely related than the beta anomer to β-D-fructose,[193] it seemed necessary to assign an α-D, α-L configuration to the D-glucosyl-L-sorboside.

The great configurational similarity between sucrose, the new D-glu-

(192) W. Z. Hassid, M. Doudoroff, H. A. Barker and W. H. Dore, *J. Am. Chem. Soc.*, **68**, 1465 (1946).
(193) C. S. Hudson, *J. Am. Chem. Soc.*, **60**, 1537 (1938).

cosyl-L-sorboside and the D-glucosyl-D-ketoxyloside was also strongly supported by the fact that all three gave a positive reaction in the Raybin color test.[194] This test depends upon the development of a blue-green color a few minutes after diazouracil (XXIII)



XXIII
Diazouracil

is dissolved in a cold alkaline solution of the sample, and on the formation of a stable blue precipitate when magnesium ion is subsequently added. The test was also positive with raffinose, gentianose and stachyose, which are tri- and tetrasaccharides including a sucrose unit,[195] but was negative for about forty other sugars, sugar alcohols and polysaccharides tried. It is interesting to note that the Raybin test was negative for isosucrose[194] and for methyl α- and β-D-fructofuranoside[140] and thus distinguished these three furanosides from members of the sucrose series. On the other hand, invertase cleaved β-D-fructofuranosides but hydrolyzed XXI and XXII very slowly if at all. The Raybin reaction therefore seems to be more specific than the action of invertase in disclosing the sucrose configuration.

(194) H. W. Raybin, *J. Am. Chem. Soc.*, **55**, 2603 (1933), **59**, 1402 (1937).

(195) C. S. Hudson, *J. Am. Chem. Soc.*, **38**, 1566 (1916); see also *Advances in Carbohydrate Chemistry*, **2**, 32–34 (1946).

BLOOD GROUP POLYSACCHARIDES

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Contents

I. Introduction

All the blood proteins of an animal can be differentiated serologically and, moreover, corresponding blood proteins of different but closely related species can also be sharply distinguished from one another. When there is qualitative overlapping between such closely related species as the dog and the wolf, or man and the anthropoid ape, the precise methods of quantitative immunochemistry readily differentiate them.

Although it is less than fifty years since Landsteiner and his coworkers showed that human beings can be divided into the so-called "blood groups," it is difficult to overestimate the important part which this knowledge has already played in blood transfusion practice. The basis of the division is, broadly speaking, the fact that human blood contains antigens ("agglutinogens") associated with the erythrocytes, and antibodies ("agglutinins") in the plasma, each of which can react with the antibodies or antigens, as appropriate, in the blood of individuals belonging to other groups. This reaction is termed "isoagglutination" and will be referred to later.

There are four main blood groups,[1] O, A, B and AB, which can be distinguished because the group-specific substances, sometimes termed "agglutinogens," A and B, may be present in the erythrocytes either singly or together, or may both be absent. In the normal human serum there are naturally present isoantibodies or "agglutinins" which can also be prepared artificially in rabbits, for example, by injection of the appropriate human erythrocytes. The A cells on injection give rise to serum containing α (or anti-A) agglutinins while the B cells likewise give β (or anti-B) agglutinins. In normal human serum these α- and β-agglutinins are regularly distributed, and in general the serum contains the agglutinin for the absent A or B factor. The combinations are shown in Table I.

TABLE I

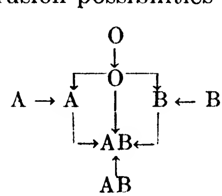
Nomenclature of whole blood	*Structure of antigen in red cells*	*Agglutinin in serum*
O	—	α (anti-A) + β (anti-B)
A	A	β
B	B	α
AB	A + B	—

In routine blood grouping, reactions occur as shown in Table II.

TABLE II

Serum	*Agglutination*			
Unknown cells + normal human B serum (α)	−	+	−	+
Unknown cells + normal human A serum (β)	−	−	+	+
Group	O	A	B	AB

Since stocks of α and β are now available, it is easy to type the blood. The significance of these reactions has been clearly set out by Boyd,[2] who summarizes the transfusion possibilities in the following diagram.



In order to avoid any reaction between the donor's cells and the recipient's serum, it is usual to transfuse from a donor in the same blood

(1) K. Landsteiner, *Wien. klin. Wochschr.*, **14**, 1132 (1901).
(2) W. C. Boyd, "Fundamentals of Immunology," Interscience Publishers, Inc., New York (1943).

group of the patient; however, in the past, donors in group O have been used as "universal" donors. In Britain and America about 45% of individuals belong to group O, 40% to A, 10% to B and 5% to AB. These percentages vary somewhat with race.

In 1910 von Dungern and Hirschfeld[3] suggested the existence of subgroups since the presence of subsidiary agglutinogens could be shown in addition to the principal ones. Agglutinogen A appears to consist of at least two separate agglutinogens, A_1 and A_2, thus giving rise to the blood groups A_1, A_2, A_1B and A_2B. Similarly agglutinin "α" is complex. No subgroups of agglutinogen B have so far been demonstrated.

There are also other blood groups quite unrelated to the A, B, O system, namely, those depending on the M and N antigens and on the rhesus (Rh) factor. The antigens M and N were discovered by Landsteiner and Levine[4] in 1928 as a result of experiments in which rabbits were injected with human bloods. The immune sera thus obtained were shown to contain other agglutinins in addition to those belonging to the then known blood groups. There was thus established the existence of three further blood groups (M, N and MN), which depended on the occurrence of these antigens. These are of importance in forensic medicine and in anthropological studies.

Another very important antigen, the rhesus (or Rh) factor, was discovered by Landsteiner and Weiner[5] in 1941 in the course of attempts to find new agglutinogens by injecting rabbits with the red cells of various animals and then testing the resulting antisera against human red cells. It was found that the sera of rabbits or guinea pigs injected with the blood of the rhesus monkey (*Macacus rhesus*), after suitable absorptive treatment to remove known agglutinins, agglutinated the red cells of about 85% of the individuals tested. Such people were then designated rhesus (Rh) +, and the agglutinogen responsible for the reaction was termed the Rh factor. It is a powerful antigen, comparable with agglutinogens A and B. Soon after its discovery in the laboratory a correlation was observed[6] between hemolytic reactions, occurring in cases of blood transfusion in post-partum cases, which could be attributed to the Rh factor and certain pregnancy complications, especially erythroblastosis fetalis (hemolytic disease of the newborn). Explanations put forward based on the properties of the Rh factor are strongly supported by statistical investigations[7] into the Rh grouping of parents of children

(3) von Dungern and Hirschfeld, *Z. Immunitätsforsch.*, **8**, 526 (1911).

(4) K. Landsteiner and P. Levine, *J. Exptl. Med.*, **47**, 757 (1928).

(5) K. Landsteiner and A. S. Weiner, *J. Exptl. Med.*, **74**, 309 (1941).

(6) P. Levine, E. M. Katzin and L. Burnham, *J. Am. Med. Assoc.*, **116**, 825 (1941).

(7) P. Levine, L. Burnham, E. M. Katzin and P. Vogel, *Am. J. Obstet. Gynecol.*, **42**, 925 (1941).

having erythroblastosis fetalis. The importance of Rh grouping is thus clearly indicated.

Isoagglutination is similar to the general agglutinin reaction familiar in immunity phenomena. As in serological reactions, so also in this case it is possible to obtain haptens capable of specifically inhibiting isoagglutination between homologous cells and sera. These haptens which can be extracted from numerous body tissues are termed "blood-group-specific substances," and it is with them that this article will be chiefly concerned. The blood group substances A, B and O have received most attention so far, owing to their more ready accessibility. The M and N antigens occur mainly in association with erythrocytes, though claims have been made[8] that they also are present in tissues. It has been stated[9] that they are soluble in organic solvents but are stable only in chloroform and ether solutions. It is not known with certainty to what extent the Rh factor occurs in cells other than erythrocytes or in secretions, but it appears to be absent from semen and to be present in saliva in only very small amounts.[8,10] Boormann and Dodd[8] state that its quantitative distribution in organs is similar to that of the A and B substances.

It was earlier thought that the blood group factors were haptens and that they could not themselves function as antigens. Various workers then showed that complete antigens could be prepared artificially and reference will be made to this work below. Kabat and his colleagues have recently shown that the factors, under appropriate conditions, specifically precipitate their homologous isoantibodies. In addition to their ability to inhibit isoagglutination, blood-group-specific substances can inhibit the hemolysis of sheep erythrocytes by rabbit sera under certain conditions. Use of this fact in assessing the activity of blood group substances has, however, largely been replaced by the isoagglutination inhibition technique, which is more reliable.[11] These two types of activity do not seem to be related in any way, inasmuch as it is possible to destroy one without interfering with the other. It has also been shown recently[12] (Section VI, page 52) that precipitin reactions can be obtained between sera containing agglutinins and the homologous group A- or B- specific substances. This reaction can serve as the basis of quantitative estimations of agglutinins. The O substances were

(8) K. E. Boormann and B. E. Dodd, *J. Path. Bact.*, **55,** 329 (1943).

(9) P. N. Kosjakow and G. P. Tribulev, *Z. Immunitätsforsch.*, **98,** 261 (1940).

(10) A. S. Weiner and S. Forer, *Proc. Soc. Exptl. Biol. Med.*, **47,** 215 (1941); P. Levine and E. M. Katzin, *ibid.*, **48,** 126 (1941).

(11) W. T. J. Morgan, *Brit. Med. J.*, **165, 2** (1944).

(12) E. A. Kabat and Ada E. Bezer, *J. Exptl. Med.*, **82,** 207 (1945).

originally tested by the difficult method of using O cells and "anti-O" sera prepared by absorbing special ox sera with A_1B cells.

A large proportion of the investigations which have dealt with this interesting group of substances have been essentially concerned with serological studies, but during the last two decades there has been a regular output of work aimed at the elucidation of their chemical nature. As yet there is but meager information concerning the chemical structure of the blood group substances, which are largely carbohydrate in character and of the mucoprotein type.

II. Blood Group Substances from Erythrocytes

The most obvious mode of approach to investigations regarding the chemical nature of agglutinogens is to examine erythrocytes, since these are assumed to contain serologically reactive substances located in the stroma. Up to the present time such studies have yielded but little in the way of satisfactory results. Schiff and Adelsberger[13] and Landsteiner and van der Scheer[14] showed that a specific substance could be extracted with alcohol from red cells. In 1934 Hallauer[15] claimed to have extracted serologically active material from human red cells of groups A, B and O by means of alcohol, but, apart from showing that in every case the active material gave positive tests for carbohydrate and that its elementary composition was of the order of C = 43 to 46%, H = 7.0 to 8.5%, N = 6.8 to 7.0% and P = 15.2%, he was able to draw no conclusions as to its chemical nature. More recently Kosjakow and Tribulev[16] claim to have isolated A, B, M and N factors from red cells. The method consisted of shaking the cells for forty-eight hours with 16% aqueous alcohol, precipitating the extraneous protein with trichloroacetic acid, dialyzing the filtrate through collodion for forty-eight hours and finally precipitating the soluble active material by means of 96% alcohol. In this way 50 ml. of washed cells yielded 5 mg. of a stable polysaccharide. So far no confirmation of these potentially important results has appeared. The present writers have attempted to obtain active blood group A material from erythrocytes by this method and numerous modifications of it, though without success. The fact that the active material of Hallauer and the other investigators was soluble in alcohol suggests a similarity to the so-called "Forsmann antigen," which will be mentioned later.

(13) F. Schiff and L. Adelsberger, *Z. Immunitätsforsch.*, **40**, 335 (1925).
(14) K. Landsteiner and J. van der Scheer, *J. Exptl. Med.*, **83**, 114 (1934).
(15) C. Hallauer, *Z. Immunitätsforsch.*, **83**, 114 (1934).
(16) P. N. Kosjakow and G. P. Tribulev, *Z. Immunitätsforsch.*, **98**, 261 (1940); P. N. Kosjakow, *ibid.*, **99**, 221 (1941).

III. Blood Group Substances from Other Sources

Fortunately from the chemists' point of view, there are polysaccharides with blood group activity more readily accessible than those from erythrocytes, and it is with the former that most chemical investigations have been concerned, although the relationship between these and the blood group substances "proper" from erythrocytes is not yet clear. Indeed the relationship may be no more than a close similarity in chemical structure of some parts of the molecular complex.

In 1926 Landsteiner and Levine[17] made the important announcement that substances possessing blood-group-serological activity occur in cells other than erythrocytes, and later supporting evidence of their presence in numerous tissues and secretions was obtained. Schiff in 1931 reported[18] that group-specific substances could be extracted from tissues in two forms, one soluble in water and another soluble in alcohol and other organic solvents. In addition to those occurring in tissues, water-soluble substances were found in most body fluids such as saliva, urine, digestive juices, cystic fluids and the like. It was discovered that not every individual secretes blood group substances in this way, so that individuals of all groups may now be divided into "secretors" and "nonsecretors." The most significant investigations reported so far have been concerned with blood-group-active material from commercial pepsin, gastric mucin, saliva and ovarian cystic fluid. Other sources which have proved useful include urine, peptone and gastric juice. The investigations described in the literature are too numerous to mention here, and since Wiener has reviewed them in his excellent book "Blood Groups and Transfusion"[19] only selected ones will be referred to in this article.

1. *From Urine*

Investigations of the serologically active material obtainable from human urine have been carried out by Freudenberg and coworkers.[20] The amount present was very small, so that urine cannot be regarded as a convenient source from which to obtain the quantities necessary for extensive investigations. These workers were able to show that the

(17) K. Landsteiner and P. Levine, *J. Immunol.*, **12**, 415 (1926).

(18) F. Schiff, "Über die gruppenspezifischen Substanzen des menschlichen Körpers," Gustav Fischer, Jena (1931).

(19) A. S. Wiener, "Blood Groups and Transfusion," C. C. Thomas, Baltimore (1943).

(20) K. Freudenberg, H. Eichel and W. Dirschel, *Naturwissenschaften*, **20**, 657 (1932); K. Freudenberg and H. Eichel, *Ann.*, **510**, 240 (1934); **518**, 97 (1935).

material from the urine of individuals of all groups was essentially a complex carbohydrate which contained *N*-acetylhexosamine and D-galactose.

2. *From Peptone*

Peptone was used as a source of blood group A material by Goebel,[21] who showed that the active substance it contained was principally carbohydrate and contained D-galactose and D-glucosamine.

3. *From Gastric Juice*

The gastric juice of "secretors" is an important source of human blood group substances, since it is of comparatively easy access and the yields of polysaccharide material obtained are reasonably high. Witebsky and Klendshoj[22] isolated active substances from the gastric juice of "secretors" of groups B and O, and have reported briefly on their properties. They appeared to be nitrogen-containing carbohydrates. We have recently shown that human gastric juice is a useful source of blood group material for chemical studies.[23] Even when comparatively drastic methods of isolation are used, for example, evaporation of solutions at 60–70° with barium or calcium carbonate, the products retain considerable activity, in some cases 0.1 mg./ml. being detectable by the isoagglutination inhibition technique. The yields are sufficiently high to make the accumulation of preparations in useful amounts possible.

4. *From Pepsin, Hog Stomach and Hog Mucin*

These have proved invaluable sources of blood group A substances, and many workers have studied the active material isolated from them. Meyer, Smyth and Palmer[24] obtained from hog stomach a polysaccharide which was highly active serologically and contained *N*-acetyl-D-glucosamine and D-galactose residues. Landsteiner and his coworkers obtained similar substances from hog stomach[25] and pig pepsin.[26] The following method used for isolating the substance from pepsin is typical.

The mucin, after filtration through a bacterial filter, was treated with a 1% solution of sodium chloride and acetic acid at 95° and the active substance was obtained by fractional precipitation with alcohol. It was then purified by reprecipitation with alcohol in the presence of sodium acetate or with acetic acid. Deproteinization by

(21) W. F. Goebel, *J. Exptl. Med.*, **68**, 221 (1938).
(22) E. Witebsky and N. C. Klendshoj, *J. Exptl. Med.*, **72**, 663 (1941); **73**, 655 (1941).
(23) H. G. Bray, H. Henry and M. Stacey, *Biochem. J.*, **40**, 130 (1946).
(24) K. Meyer, E. Smyth and J. Palmer, *J. Biol. Chem.*, **119**, 73 (1937).
(25) K. Landsteiner and R. A. Harte, *J. Exptl. Med.*, **71**, 551 (1940).
(26) K. Landsteiner and M. W. Chase, *J. Exptl. Med.*, **63**, 851 (1936).

Sevag's method,[27] using chloroform and butyl alcohol, has also been used for purification, the purification process being followed by the isoagglutination method.

A detailed investigation of the material from hog stomach has recently been reported by Kabat and his coworkers.[28] Reference is made to it later in this article, and also to the studies of Morgan and King[29] which deal with the blood group substance from hog mucin. The latter workers have developed useful methods for obtaining these substances in a relatively undegraded condition. One method involved the precipitation of the serologically active material from a concentrated aqueous solution of mucin by the addition of anhydrous sodium sulfate. The blood group A substance was precipitated sharply by 27–30% sodium sulfate and purified by further precipitations in a similar manner and finally dissolved in water, dialyzed at 0° and then dried in a vacuum from the frozen state. A second method made use of the fact that 90% phenol will dissolve the blood group A substance present in crude mucin, leaving behind insoluble denatured protein. Repeated extraction with phenol and precipitation by means of alcohol gave a very active substance which was isolated by solvent precipitation as in the first method.

The present writers have used commercial pepsin as a source of active material, which is being studied by methylation methods. Our preliminary findings have already been reported,[30] while our more recent results will be discussed below. The polysaccharide residue was isolated by the action of barium hydroxide solution on pepsin, the yield of crude material being 15–20%. Methylation of this product gave material having a methoxyl content of 32% and $[\alpha]_D - 20°$ in water. The yield of the methyl ether was about 15% by weight of the crude polysaccharide. The presence in the preparation of derivatives of D-galactose, D-glucosamine, L-fucose and D-mannose was shown by the usual hydrolysis methods.

5. *From Pseudomucinous Ovarian Cyst Fluids*

King and Morgan[31] obtained a very active blood group substance from the cystic fluids of individuals of group A and were able to show that it was very similar in properties to the substance they obtained from hog mucin. More recently Morgan and Waddell[32] have reported the isolation of the corresponding O material from group O cystic fluid. The sub-

(27) M. G. Sevag, *Biochem. Z.*, **273**, 419 (1934).

(28) E. A. Kabat, A. Bendich and Ada E. Bezer, *J. Exptl. Med.*, **83**, 477, 485 (1946).

(29) W. T. J. Morgan and H. K. King, *Biochem. J.*, **37**, 640 (1943).

(30) H. G. Bray, H. Henry and M. Stacey, *Biochem. J.*, **40**, 124 (1946).

(31) H. K. King and W. T. J. Morgan, *Biochem. J.*, **38**, x (1944).

(32) W. T. J. Morgan and M. B. R. Waddell, *Brit. J. Exptl. Path.*, **26**, 387 (1945).

stances of the two groups appear to be very similar in the properties studied, differing mainly in their specific rotations (see Table III, p. 46).

6. *From Saliva*

The saliva of "secretors" has been used as a source of blood group substances in several investigations. Landsteiner[33] obtained very active material from horse saliva and was able to show that it contained D-galactose and a hexosamine. More recently the same author compared the blood group substances from the saliva of individuals of groups A, B and O[34] and found that there was very little difference in their properties (see Table III).

IV. Properties and Chemistry of Blood Group Substances

Although no complete investigations of the structure of the blood group substances have yet been reported, it is evident from the information available that all the material so far studied is predominantly carbohydrate in nature, and, as can be seen from Table III, preparations from widely different sources have many properties in common, for example, elementary analysis, specific rotation and the like. It is of interest to note that almost all the substances obtained from nonhuman sources display blood group A or blood group O activity, while human material is the most convenient source of the blood group B substance. The majority of the work carried out so far has dealt with blood group A substances.

It will be observed that in the cases in which constituent sugar units have been identified, D-galactose and *N*-acetyl-D-glucosamine have frequently been found. In addition, we have shown[30] the presence of L-fucose in appreciable amounts in the active material from commercial pepsin. D-Mannose was also found in small amounts, but it is not yet certain whether this sugar is present as a true constituent of the active polysaccharide. The blood group A carbohydrate material was methylated using methyl sulfate and sodium hydroxide (35%), followed by methyl iodide and silver oxide. The fully methylated material (30.8% methoxyl) was hydrolyzed with 2% methyl alcoholic hydrogen chloride and from the resulting mixture the methyl ethers of L-fucose, D-glucosamine, D-galactose and D-mannose were isolated, the last two as anilides. The arrangement of the sugar units within the carbohydrate itself is still uncertain. The methylated polysaccharide after hydrolysis was separated into two fractions, one soluble and the other insoluble in ether. The methyl ethers of the four sugars mentioned above were all shown

(33) K. Landsteiner, *J. Exptl. Med.*, **63**, 185 (1936).
(34) K. Landsteiner and R. A. Harte, *J. Biol. Chem.*, **140**, 673 (1941).

TABLE III

Properties of Blood Group Substances from Various Sources

Source	Investigators	[α]D	N, %	Acetyl, %	C, %	H, %	Amino acid N, %	Reducing sugar (on hydrolysis), %	Hexosamine, %	Constituent sugars identified
Erythrocytes	Hallauer (15)		6.8–7.0		43–45	7.0–8 5				
Urine	Freudenberg *et al.* (20)	Levo	5	9.1				45 5		*N*-Acetyl-D-glucosamine, D-Galactose
Peptone (Difco) (Pfanstiehl)	Goebel (21)	+11.5° +9.6	5 85 5.48	9 56 8 9	46.7 44.9	6 53 6 23		73 62		D-Galactose D-Glucosamine
Pepsin (pig)	Landsteiner and Chase (26)	+16.0	6.2	10 0	46.9	6.6		70 7		
Hog mucin	Meyer *et al.* (24)	−8.0–+8.7°	3 3–6.2	9 2–16 8					30 8–38 6	D-Galactose, *N*-Acetyl-D-glucosamine
Hog stomach	Landsteiner and Harte (25)	+19.8–+21.9°	5.5–6.3	10.5–12 0	46–47	6 6–6.9		50–60	29–33	
	Kabat *et al.* (28) (a)		5 6–6.8	9.1–11.3				46–59	25–32	L-Fucose, D-Galactose,
	(b)		5 7–6.6					55–61	30–34	D-Glucosamine
Hog mucin	Morgan and King (29)	+10.0[a]	6 0	10.0	45	6 5	2 2	50	27	
Gastric juice (Human group B)	Witebsky and Klendshoj (22)		1 5–1 6					75		
Gastric juice (Human group O)	Witebsky and Klendshoj (22)		2 8					39.8		
Cyst fluid (Human group A)	King and Morgan (31)	+11.0[a]	6 0	10 0	44–45	6 7	2.5	50	27	
(Human group O)	Morgan and Waddell (32)	−23.0[a]	5 9		44.5	6 6	2 5	48	38	
Saliva (Horse)	Landsteiner (33)	+10.0	7 1	9 4	44 6	6 9		56–58		D-Galactose, Hexosamine
Saliva (Human group A B O)	Landsteiner and Harte (34)		5 65 5 33 5 74				2.48 2.35 2 91	45 5 48 5 46.5	23.3 21.7 21.5	
Type XIV pneumococcus polysaccharide	Goebel *et al.* (41)	+6.5–+12.6	1.91–2.08	6 6–7 7	44.98	6.46		84.4		*N*-Acetyl-D-glucosamine, D-Galactose

[a] 7607 Å.

to be present in the ether-soluble fraction. Part of the L-fucose was isolated directly as methyl 2,3,4-trimethyl-α-L-fucoside, indicating that it was present originally as an end group. The presence of partially methylated L-fucose derivatives as intermediate links in the molecule was also shown by the fact that, after further methylation of portions of the ether-soluble fraction from which the fully methylated L-fucose end residue had been removed, further yields of derivatives of this sugar were obtained. Neither D-galactose nor D-mannose could be isolated from the hydrolyzate of the original methyl ether as fully methylated derivative, and treatment of these substances with silver oxide and methyl iodide was necessary before they could be converted to their crystalline 2,3,4,6-tetramethyl-anilides for identification. Part of the D-glucosamine derivative isolated from the ether-soluble fraction was identified as methyl *N*-acetyl-3,4,6-trimethyl-D-glucosaminide, indicating that some of the D-glucosamine residues are linked as terminal groups. It seems probable that the proportion of amino sugar linked in this way is small, for the majority of it appears to be located in the ether-insoluble fraction. This may constitute a type of polyglucosamine "core," possibly resembling a methylated chitin. Its molecular weight appears to be of the order of 800–900 and it contains 5% nitrogen. Its acetyl content cannot be raised above 2% by repeated treatment with acetic anhydride and methyl alcohol at room temperature, a procedure which the writers have found to acetylate only amino groups. Treatment with acetic anhydride and sodium acetate at 140° gave a small yield of a sirup having 14.8% acetyl residues. The methoxyl content of the original stable polyglucosamine derivative was 24.9% and after repeated methylation the content rose to 27.1%. Attempts to hydrolyze the polyglucosamine constituent with 5% methanolic hydrogen chloride, with hydrochloric acid of strengths up to 10 *N* at 100°, with fuming hydrochloric acid at room temperature and with 10 *N* hydrochloric acid at 120–130° all gave polymeric products in the form of colorless, viscous resins having no reducing properties. It was noted however that by this treatment considerable demethylation occurred, the methoxyl content in some instances being as low as 5%.

While it is impossible to make any precise statement regarding the structure of this blood group A polysaccharide until our present investigations are more advanced, it does seem possible that the structure of the alkali-stable carbohydrate residue is of a ramified type, bearing some general relationship to that deduced for ovomucoid,[35] which, however, is much less resistant to hydrolysis than is this blood group A polysaccharide.

(35) M. Stacey and J. M. Woolley, *J. Chem. Soc.*, 184 (1940); 550 (1942).

It is of interest to note that we have obtained the same fully methylated L-fucose derivative from Morgan's hog mucin preparation[29,30] (see p. 44) and it is indeed probable that the group substances from pepsin and hog mucin are identical in many details of their structure.

The writers' investigations on the blood group substances from human gastric juice have already been indicated.[23] It now seems possible that the polysaccharide isolated from the gastric juice of group A humans may be different from the pepsin polysaccharide, inasmuch as the methyl ethers which we have recently obtained from them have different specific rotations. The work referred to above has as its main object the elucidation of the structure of the stable polysaccharides which form part of the molecular structure of the blood group substances as they occur in the natural state. Morgan and his coworkers have studied in some detail the properties of preparations made by using the mild conditions of isolation with phenol already referred to.[29,31,32] These substances may be regarded as being very closely related to, if not identical with, the blood group substances as they actually occur in secretions and body fluids. They retained much of the viscosity of the original mucin, and it was possible to correlate this property with the ability to inhibit isoagglutination. These authors found that treatment with 0.1 *N* sodium carbonate at 100° caused some degradation of the molecule, the serological activity being reduced to 1% of the original value and the specific rotation changing from $[\alpha]_D + 10°$ to $-20°$. This change was accompanied by the splitting off of some of the hexosamine residues, which were separated by dialysis and shown to contain free reducing groups. A residual nondialyzable fragment retained reducing properties.[36] It would appear that some of the hexosamine constituents are linked to the remainder of the complex through position 1 and that this link is very unstable toward dilute alkali.

Kabat and coworkers[28] have recently reported a careful study of the properties of blood group substances prepared by Morgan's phenol method[29] from hog stomach linings. In these investigations the relative purity of various preparations was determined, using the valuable microquantitative precipitin method referred to earlier,[12] that is, by comparing the quantities of each preparation that are necessary to precipitate a given amount of the isoantibody. He showed that the blood group substance was serologically stable for two days at 37° between *p*H 1.07 and 10.7, and that heat treatment for two hours at 100° did not cause any loss of activity if the *p*H was between 2.97 and 7.58. Inactivation did occur at 100° at *p*H values below 1.03 or above 9.03.

(36) W. T. J. Morgan, *Brit. Med. Bull.*, **2**, 165 (1944).

This confirms Morgan's findings[36] regarding the alkali lability of these carbohydrates. A study of the blood group substances obtainable from individual hog stomach linings revealed that three out of ten gave products which had no blood group activity, although all ten had identical chemical properties. While, as described later, it was concluded that the constancy of analytical properties gives no information as to their purity and activity, it is nevertheless remarkable that products from so many different sources, prepared by several methods and by many workers, should display such a similarity in chemical properties and it is clear that blood group activity must be associated with some relatively small fragment of the molecular pattern. Kabat and his coworkers[28] devised a method for assessing the absolute purity of their preparations by correlating the D-glucosamine content of the substance precipitated by isoantibody with the total amount present in the particular preparation. They concluded that their preparations were about 84% pure.

In connection with the apparent lack of correlation between precise chemical properties and blood group activity it is interesting to note that Witebsky and Klendshoj[22] found no relationship between the reducing sugar contents of their preparations from human gastric juice and the isoagglutinin activity.

It is now recognized that amino acids are important constituents of the blood group substances. Landsteiner and Harte[25] showed that the amino acid nitrogen content accounted for the bulk of the nonhexosamine nitrogen present, and they were the first to suggest that amino acids play a part in the serological specificity of these haptens. It was then realized that the slightly positive protein color reactions given by all the known preparations must be due to these amino acids and not necessarily to small amounts of protein impurity. Some detailed investigations of the amino acid content of various preparations have now been reported. Thus Freudenberg, Walsh and Molter[37] isolated threonine from the blood group A substance and Morgan reports that, using paper chromatography, at least fifteen amino acids, including threonine and hydroxyproline in high concentrations, have been found. Cystine appears to be absent.[36] In an appendix to the second of the papers by Kabat and his colleagues,[28] Brand and Saidel have reported the presence in the hog stomach blood group A substance, of glycine (1.6%), valine (0.7%), isoleucine (0.3%), proline (3.3%), phenyalanine (0.1%), tryptophane (0.2%), histidine (0.6%), lysine (1.0%), aspartic acid (0.8%), glutamic acid (1.3%), serine (1.9%) and tyrosine (0.3%).

Most of the work referred to concerns blood group A substances.

(37) K. Freudenberg, H. Walsh and H. Molter, *Naturwissenschaften*, **30**, 87 (1942).

Little has yet been reported on the chemical properties of blood group B and O substances; Table III (page 46) summarizes the information thus far obtained.

Some details of the structure of these interesting substances are now gradually being revealed, but, until the precise mode of linkage of the sugar units with the amino acids and of the sugar units themselves in the polysaccharide portion of the molecule are understood, it will be impossible to integrate all the results obtained and difficult to arrive at generally applicable conclusions as to the factors which determine group specificity and degree of activity. There is need for a concerted effort directed toward the chemical approach to the problem, for in addition to elucidating the structure of individual blood group substances, investigations of this kind may reveal the causes underlying specificity itself. It is tempting to speculate that the specific substances of all the groups may contain the same type of "core" structure—possibly mainly polysaccharide—and that the attached amino acids are responsible for determining group specificity (see Section V, below). We have observed that several naturally occurring polysaccharides, for example, frog spawn mucin and damson gum, may exhibit to some degree the ability to inhibit isoagglutination[30] of the blood group A substance. It will be of interest to determine precisely what groupings are responsible for blood group activity and what is the role of the carbohydrate–amino acid complexes in this connection. Compounds of this type have not yet been studied to any appreciable extent, and it is possible that investigations of this nature might yield important results applicable to the whole field of protein chemistry.

V. Naturally Occurring Polysaccharide Complexes of Interest in Connection with Blood Group Substances

1. *The Forsmann Antigen*

During the course of investigations by several workers it was noted that blood group substances had some properties very similar to those of other naturally occurring complex carbohydrates. The fact that blood group substances from some sources, especially those from erythrocytes, could be isolated by extraction with alcohol, suggested a general similarity to the so-called Forsmann antigen. This is a hapten found in many mammalian tissues and in some bacteria, for example, *Bacillus shigae.* When injected into some animals, for example rabbits, it gives rise to "immune" sera which can hemolyze sheep blood. It is usually regarded as a type of lipid hapten, but whether the lipid residue is an essential part of its structure is uncertain. Moreover, very active preparations can

be obtained[38] which are insoluble in alcohol, though they do contain fatty acids. Landsteiner and Levene[38] and Brunius[39] found that certain lipids would increase the activity of purified preparations of the hapten and cause them to react with homologous antisera. Chemical investigations of the Forsmann antigen from horse kidney[38,39] have shown that on hydrolysis it gives rise to both reducing sugars and fatty acids. Brunius states that *N*-acetylhexosamine is an essential part of the molecule. It is interesting to note its similarity to some bacterial polysaccharides,[39a] for example, those from *Mycobacterium tuberculosis.*[39b]

2. *The Type XIV Pneumococcus Polysaccharide*

The specific polysaccharide of type XIV pneumococcus[39a] is of particular interest, for the immune sera obtained by the injection of this polysaccharide agglutinate human erythrocytes and precipitate the blood group A substances. Beeson and Goebel[40] have carried out a detailed investigation of the immunological relationships between the pneumococcus type XIV polysaccharide and the blood group A substance from peptone.[21] They showed that the agglutination of human red cells by type XIV antipneumococcus horse serum is a typical serological cross reaction and appears to be due to similarities in the chemical constitution of the two polysaccharides. The authors point out their strikingly similar properties for, as mentioned above, the blood group A polysaccharide contains both D-galactose and *N*-acetyl-D-glucosamine, and Goebel, Beeson and Hoagland[41] have shown that the pneumococcus type XIV polysaccharide also contains D-galactose and D-glucosamine in the ratio 1:3. Moreover, these substances resemble each other in optical rotation, elementary analysis and in their precipitation reactions with salts of heavy metals (see Table III). It would seem that the main difference between them is the important fact that the type XIV polysaccharide does not contain bound amino acid residues.

Agglutinin and precipitin cross reactions also occur between blood

(38) K. Landsteiner and P. A. Levene, *J. Immunol.*, **14**, 81 (1927); *Proc. Soc. Exptl. Biol. Med.*, **24**, 693 (1927).

(39) F. E. Brunius, "Chemical Studies on the True Forsmann Hapten, the Corresponding Antibody and Their Interaction," Thesis, Stockholm (1936) (quoted by Wiener[19]).

(39*a*) See M. Stacey, *Advances in Carbohydrate Chem.*, **2**, 162 (1946); T. H. Evans and H. Hilbert, *ibid.*, 204.

(39*b*) See M. Stacey and P. W. Kent, *Advances in Carbohydrate Chem.*, **3**, 311 (1948).

(40) P. B. Beeson and W. F. Goebel, *J. Exptl. Med.*, **70**, 239 (1939).

(41) W. F. Goebel, P. B. Beeson and C. L. Hoagland, *J. Biol. Chem.*, **129**, 455 (1939).

group A substances and the antibodies corresponding to pneumococcus type I and *Salmonella schottmülleri*.

Another aspect of the same problem has already been referred to.[30] We have shown that several naturally-occurring polysaccharides show a slight blood group A activity, the main ones being ovomucoid, frog spawn mucin and damson gum. Ovomucoid contains D-mannose, D-galactose and *N*-acetyl-D-glucosamine[35] and may resemble the blood group A substance from pepsin somewhat in structure, but damson gum would appear to be of a very different nature. Hirst and Jones[42] have investigated this polysaccharide in detail and find it to contain L-arabinose, D-xylose and D-glucuronic acid in addition to D-galactose and D-mannose. It contains no nitrogen. The polysaccharide of frog spawn mucin has not been thoroughly studied, but we have found that it contains nitrogen, a part of which is probably present in the form of hexosamine, and D-galactose. Much more detailed information about the constitution of these and similar carbohydrates is necessary before any conclusions can be drawn about the relationship, if any, between chemical constitution and blood group activity. Few of these polysaccharides have as yet been tested for blood group O activity and this will now be of some interest.

VI. Antigenic Properties of Blood Group Substances

Although blood group A and B substances give a remarkably specific inhibition of the isoagglutination reaction, they have not been known to give the precipitin reaction with human sera containing homologous isoagglutinins.

The blood group A and B substances were recently shown to be antigenic in man[43] and both give high-titer antisera. Kabat, Bendich and Bezer[12,28] have applied the valuable quantitative precipitin technique of the Heidelberger school for the estimation of A and B isoantibodies in human serum. These workers showed that on injection of purified blood group A substances into individuals of blood groups B and O there were produced significant amounts of precipitin in the sera, whereas the injection of individuals of blood groups A and AB with the same A preparation gave no precipitins.

With individuals of blood group O, injection of A and B substances gave a big increase in the amounts of precipitins. For example, the specific antibodies to the blood group A substance could be obtained from the specific precipitate by the use of 15% sodium chloride for dissociating

(42) E. L. Hirst and J. K. N. Jones, *J. Chem. Soc.*, 1174 (1938); 1482 (1939).

(43) E. Witebsky, N. C. Klenshoj and C. McNeil, *Proc. Soc. Exptl. Biol. Med.*, **55**, 165 (1944).

it. The quantitative aspects of the work showed that the reactions of the blood group substances with their homologous immune sera were the same as in other antigen–antibody systems.

Quantitative immunochemical methods[44] appear to be valuable for distinguishing between blood group A substances and blood group A_1 and A_2 substances from different sources, especially in regard to their purity as determined by chemical properties. The glucosamine content of the specific precipitate appears to give a rapid method of assaying the purity of blood group substances.

The importance of a knowledge of the source of blood group substances was strikingly demonstrated by Morgan and his colleagues,[45] who examined the blood group substances from twenty-four individual hog stomachs by chemical and serological methods; fourteen possessed blood group A specificity only and ten possessed O specificity only. Most commercial preparations of blood group A substance from pooled hog stomach material, even though electrophoretically homogeneous, possess both blood group A and O activity, while substances prepared from human ovarian cysts have no O activity.

This work on individual hog stomach material has been extended by Kabat and his colleagues,[46] who obtained substances showing either A or O specificity or both A and O specificity in varying proportions. An important finding was the confirmation of previous work[47] which claimed that the Forsmann antigenic activity was associated with blood group A activity only. The purified blood group A and O substances were identical in regard to their contents of nitrogen, reducing sugar, glucosamine and acetyl residues and also with respect to their relative viscosity and electrophoretic mobility. The blood group O substance as well as the blood group A substance contained L-fucose, D-glucosamine and D-galactose residues, and it was concluded that only immunochemical methods can give a true assay of the purity of a given preparation.

VII. Artificial Antigens from Blood Group Substances

It has already been stated that, prior to the work discussed in Section VI, it was generally considered that blood group substances were not complete antigens but haptens only. The possibility of converting them into true antigens in a manner similar to that by which bacterial polysac-

(44) E. A. Kabat, A. Bendich, Ada E. Bezer and S. M. Beiser, *J. Exptl. Med.*, **85**, 685 (1947).

(45) D. Aminoff, W. T. J. Morgan and W. M. Watkins, *Nature*, **158**, 879 (1946).

(46) A. Bendich, E. A. Kabat and Ada E. Bezer, communication from E. A. Kabat.

(47) F. Schiff and L. Adelsberger, *Z. Immunitätsforsch.*, **40**, 335 (1924); *Centr. Bakt. Parasitenk. Orig.*, **93**, 172 (1924).

charides have been converted into antigens has been investigated by various workers, notably by Morgan and his colleagues.

Avery and Goebel[48] showed that antigens with a new immunological specificity could be made by linking phenyl β-D-glucoside and phenyl β-D-galactoside to horse serum globulin or to egg albumin, using a diazotization method. The main new specificity of these artificial antigens depended essentially on the carbohydrate residue, for those formed from the same carbohydrate with different proteins had a common specificity, while those from different carbohydrates and the same protein had different specificities. In this work a reaction similar to the inhibition of isoagglutination was also observed. Thus the unconjugated phenyl glycosides were found to inhibit the reaction between the homologous synthetic antigen and its antibody. Later these workers prepared an artificial antigen from the specific polysaccharide of type III pneumococcus with serum globulin and showed that it could confer specific immunity when injected into rabbits and mice.[49]

Partridge and Morgan[50] have carried out similar experiments in which various polysaccharides were converted into antigens by being coupled with the purified protein component of the O somatic antigen of *Bacillus shigae* or *B. typhosum*. Among the polysaccharides used were agar, gum acacia and cherry gum, and antigens were obtained having properties generally similar to those prepared by Goebel and Avery.[50] Using the blood group A substance from hog mucin, Morgan was able to prepare an artificial antigen of the same type by mixing aqueous solutions of the bacterial protein and the polysaccharide.[36] On injection into rabbits this antigen gave rise to potent anti-blood-group-A immune sera.

Westphal, Reiche and Krah[51] have coupled a blood group A substance with egg albumin using the method of Avery and Goebel.[48]

The antisera to which these artificial antigens give rise on injection are of considerable importance in blood grouping. Witebsky and his colleagues[52] have introduced an important innovation in blood transfusion technique based on the knowledge that haptens can inhibit specific reactions. They have suggested that the specific blood group factors be added to group O samples ("universal donor type") in order to neutralize any isoagglutinins, before intergroup transfusion. Clinical studies have

(48) O. T. Avery and W. F. Goebel, *J. Exptl. Med.*, **50**, 521, 523 (1929)

(49) W. F. Goebel and O. T. Avery, *J. Exptl. Med.*, **54**, 431, 437 (1931).

(50) S. M. Partridge and W. T. J. Morgan, *Brit. J. Exptl. Path.*, **23**, 84 (1942).

(51) O. Westphal, E. Reiche and E. Krah, *Naturwissenschaften*, **32**, 40 (1944).

(52) E. Witebsky, N. C. Klendshoj and P. Swanson, *J. Am. Med. Assoc.*, **116**, 2654 (1941); N. C. Klendshoj, C. McNeil, P. Swanson and E. Witebsky, *Arch. Internal Med.*, **70**, 1 (1942).

shown the value of this practice, and purified blood group A and B factors are available commercially for this absorption purpose.

VIII. Destruction of Blood Group Substances by Specific Enzymes

Schiff[53] showed that filtrates and cultures from some strains of *Clostridium welchii* inactivated the blood group substances from peptone and saliva and the action of *Cl. welchii* (type A) culture filtrates on blood group substances was recently investigated.[54] The enzymes present included collagenase and hyaluronidase as well as "α and θ" toxins. Incubation with the enzyme at 37° destroyed activity as measured by isoagglutination inhibition, but not as measured by the hemolysis inhibition. When the enzyme preparations were previously heated to 56°, gastric mucin blood group A substance and human cyst blood group A and B substances were not affected, but hog and human blood group O substances were destroyed. Thus the preparation seemed to contain two enzymes, one thermolabile, attacking blood group A and B substances and one thermostable, attacking the blood group O substance. It was noted that *Cl. welchii* antiserum prevented the destruction of blood group A and B substances but not the blood group O substance. *Cl. welchii* hyaluronidase preparations had no action on blood group A and B substances, but attacked the blood group O substance. Preparations of "α and θ" toxins had the same effect as hyaluronidase, and the action was not inhibited by the corresponding antitoxins; it was therefore concluded that the action was not due to toxins themselves. It is of interest to note that during the enzyme action, solutions of the blood group substances became levorotatory, their reducing power increased, their viscosity decreased and certain amino acids were liberated. Future work along these promising lines is awaited with interest.

(53) F. Schiff, *Klin. Wochschr.*, **14**, 750 (1935); *J. Infectious Diseases*, **65**, 127 (1939).

(54) W. T. J. Morgan, *Nature*, **158**, 759 (1946).

APIOSE AND THE GLYCOSIDES OF THE PARSLEY PLANT

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CONTENTS

I. INTRODUCTION

The parsley plant (French *persil*, German *Petersilie*) contains in its seeds (fruit?), stems and leaves at least two glycosides, the aglycons of which are two differently substituted flavones. The carbohydrate portion of the usually predominating glycoside is composed of D-glucose and a remarkable branched-chain aldopentose which was named apiose by its discoverer (Vongerichten) from the Linnean designation of the parsley plant, *Apium petroselinum*. The second glycoside, which usually occurs in smaller proportion, probably also carries these two sugars in its structure but the evidence for this view is so indirect that it

obviously requires substantiation. The reason why there exists better knowledge of the first glycoside lies in the fact that many of the samples of parsley glycosides which were the subjects of the investigations consisted largely of it, while those from which our knowledge of the second glycoside has been obtained were mixtures of the two glycosides containing probably never more than about fifty percent of the second glycoside. The two glycosides have never been fully separated and studied as pure chemical individuals. The crystalline mixture of glycosides from parsley, consisting principally or possibly wholly of these two glycosides, was believed in early days to be a single entity and the name *apiin* was given to it by the investigator (Lindenborn) who was the first to obtain the material in crystalline form. This name has become restricted later to the predominating glycoside which, though never yet isolated as a single entity, is known almost certainly to be composed of one molecule each of apiose, D-glucose and 5,7,4′-trihydroxyflavone, the substituted flavone being attached at its position 7 to the D-glucose molecule by glycosidic union and the apiose molecule being attached at some unknown position in the D-glucose moiety by a disaccharide union (see page 72). The conclusion has been stated, especially in Vongerichten's numerous researches in this field, that the second glycoside of parsley is actually the 3′-hydroxy-4′-methyl ether of the first glycoside but the evidence at the present time does not seem to the writer to be sufficiently precise to justify acceptance of this view as other than a hypothesis. It is established that the aglycon of this second glycoside is 5,7,3′-trihydroxy-4′-methoxyflavone (diosmetin (XIII)) but the locating of the position of the glycosidic union and a proof of the precise structures and anomeric forms of the components of the carbohydrate moiety can hardly be expected in advance of the isolation of the glycoside in an essentially pure form. The early literature on the parsley glycosides is confusing to the modern reader because the names apiin and apigenin were used in varying meanings as time passed, and also because some of Vongerichten's articles state in the form of established conclusions matters that were really only doubtful inferences at the time. Some of these inferences proved to be unsound but it is to Vongerichten's credit that in many of them he showed a remarkable prescience; a just evaluation of his researches requires recognition of the fact that the period during which he studied the parsley glycosides (1876–1906) was the one during which there was arising, but only gradually, the modern chemistry of the carbohydrate and flavone groups.

The writer believes that clarity in the present review will be greatly aided if an initial statement of some definitions be made; also it seems necessary to introduce a few names to replace some designations that

were used by Vongerichten but are now believed to be inappropriate or confusing.

II. Definitions of the Names Apiin, Petroselinin, Apigenin and Diosmetin

Apiin. This name will be restricted to apply to an assumed chemical entity that is the main glycosidic constituent of the seed (fruit?) of parsley. It also occurs in the leaves and stems but the "crude apiin" from them contains sometimes as much as fifty percent of the second glycoside.[1] It has never been isolated entirely free of the accompanying second glycoside but the evidence from studies of material that consisted mostly of apiin appears to justify the definition that its molecule is composed of one molecule of 5,7,4′-trihydroxyflavone, one of D-glucose and one of apiose. The mixture of glycosides that has borne the name of "apiin" in the literature will be referred to as "crystalline parsley glycosides" or as "crude apiin."

Petroselinin. This is a new trivial name which will designate as an assumed chemical entity the second glycoside of parsley. Thus "crude apiin" signifies at present a mixture that consists primarily of apiin and petroselinin; it is of course possible that other substances may also be present in small proportion. Vongerichten[1] inferred that apiin and petroselinin differ only in respect to their aglycons and accordingly he gave the name "monomethyl ether of hydroxyapiin" to the assumed glycoside that is here termed petroselinin. Although his inference from meager evidence that petroselinin is a substituted apiin (the substitutions being only in the aglycon) may eventually be shown to have been correct, there is no proof at present that the relationship between apiin and petroselinin is such a close one. It seems best to avoid at present the name which implies this unproved relationship and to use instead the trivial name petroselinin for the second crystalline parsley glycoside.

Apigenin. This name will be restricted to the pure substance which v. Kostanecki and coworkers[2] proved through its synthesis to be 5,7,4′-trihydroxyflavone (VI, page 63); they found that the synthetic product is identical with the principal component of the natural "crude apigenin." The materials that are designated "apigenin" in the prior literature are now presumed to have been a mixture of true apigenin with varying smaller amounts of diosmetin. True apigenin is the aglycon of true apiin, the predominating glycoside of parsley.

Diosmetin. After v. Kostanecki and coworkers[2] isolated true apigenin from "crude apigenin" and proved its identity with the syn-

(1) E. Vongerichten, *Ber.*, **33**, 2334 (1900).
(2) J. Czajkowski, S. v. Kostanecki and J. Tambor, *Ber.*, **33**, 1988 (1900).

thetic 5,7,4′-trihydroxyflavone, Vongerichten[1] succeeded in isolating a second aglycon from "crude apigenin"; he proved that it is a monomethyl ether of luteolin (5,7,3′,4′-tetrahydroxyflavone) and obtained evidence which made it probable that the substance is 5,7,3′-trihydroxy-4′-methoxyflavone. Oesterle and Wander[3] ascribed this structure also to the aglycon of a rhamnoglucoside that they found to be widely distributed among plants; they adopted for this rhamnoglucoside the earliest of its various trivial names, diosmin, and named its aglycon diosmetin.[4] Finally, the syntheses of 5,7,3′-trihydroxy-4′-methoxyflavone and 5,7,4′-trihydroxy-3′-methoxyflavone by Robinson and coworkers[5] conclusively established the first of these structures as that of diosmetin (XIII, page 65) and of Vongerichten's "luteolin monomethyl ether" as well. The aglycon of petroselinin is thus the same as the aglycon of diosmin, but petroselinin cannot be the same glycoside as diosmin since Votoček[6] has shown that "crude apiin" gives a negative test for methyl pentoses and therefore cannot contain a rhamnoglucoside.

III. Early Knowledge of Parsley and Its Glycosides

The following quotation concerning the origin of the cultivated parsley plant is taken from an English translation of the 1882 French edition of DeCandolle's well-known book.[7]

> "Parsley—*Petroselinum sativum*, Moench. [*Apium petroselinum* L]. This biennial Umbellifer is wild in the south of Europe, from Spain to Turkey. It has also been found at Tlemcen in Algeria, and in Lebanon. Dioscorides and Pliny speak of it under the names of *Petroselinon* and *Petroselinum*, but only as a wild medicinal plant. Nothing proves that it was cultivated in their time. In the Middle Ages Charlemagne counted it among the plants which he ordered to be cultivated in his gardens. Olivier de Serres in the sixteenth century cultivated parsley. English gardeners received it in 1548. Although this cultivation is neither ancient nor important, it has already developed two varieties, which would be called species if they were found wild; the parsley with crinkled leaves, and that of which the fleshy root is edible."

(3) O. A. Oesterle and G. Wander, *Helv. Chim. Acta*, **8**, 519 (1925).

(4) The name diosmin was introduced by R. Brandes (*Arch. Pharm.*, [1] **22**, 224 (1827) (Army Medical Library, Washington)) in his description of an amorphous material from an extract of buchu leaves (*Barosma* (= *Diosma* L) species). It was retained by X. Landerer (*Buchner's Repertorium für die Pharm.*, [2] **34**, 63 (1844) (Army Medical Library, Washington)) who applied it to a crystalline substance from such extracts; the crystals were soluble in ether and therefore probably were not the glycoside. The name became established as that of the pure crystalline glycoside of buchu leaves through the research of P. Spica (*Gazz. chim. ital.*, **18**, 1 (1888)).

(5) A. Lovecy, R. Robinson and S. Sugasawa, *J. Chem. Soc.*, 817 (1930).

(6) E. Votoček, *Z. Zuckerind. Böhmen*, **24**, 239 (1900).

(7) Alphonse P. DeCandolle, "The Origin of Cultivated Plants," International Scientific Series, Appleton and Co., New York, 1902.

A hot aqueous extract of parsley seed or plant yields, after cooling, a gelatinous material which was first mentioned by Rump.[8] It was later studied by Braconnot,[9] who compared it to pectin in its behavior and gave it the name apiin. He found that its hydrolysis by acid liberated material which reduced Fehling solution, as did pectin after hydrolysis. Von Planta and Wallace[10] described many characteristics of the products which they obtained from the acid hydrolysis of gelatinous apiin; one of these, a sirup, reduced Fehling solution but they were not certain that it contained a "glucose" because it did not crystallize and they were unable to ferment it with yeast. Another product was a flocculent material which was nearly insoluble in cold water; present information leads one to believe that this material was a very impure sample of the "crude apigenin" of later investigators.

It was Lindenborn[11] who first obtained the mixture of parsley glycosides in a crystalline condition. The acid hydrolysis of this more definite material yielded a crystalline substance to which he gave the name apigenin on the supposition that it was a chemical entity. Such crystalline "crude apiin" and "crude apigenin" were the objects of a long series of investigations by Vongerichten, beginning in 1876, which led him to the discovery of apiose and eventually to the recognition of the unusual branched-chain structure of this pentose.

IV. Investigations of "Crude Apigenin"

In 1876 Vongerichten[12] fused "crude apigenin" with caustic potash and identified phloroglucinol, protocatechuic acid and *p*-hydroxybenzoic acid among the products. Knowing the carbon, hydrogen and oxygen content of "crude apigenin," which he considered to be a pure chemical compound, he inferred from the identified fusion products that the formula of the substance was $C_{15}H_{10}O_5$, which is indeed the correct formula that is now known for pure apigenin. This formula is an example of several fortunate inferences that Vongerichten made from data of questionable value which he obtained in the course of his researches on the parsley glycosides.

Twenty-one years elapsed after Vongerichten's early study of "crude apiin" and "crude apigenin" before the subject received further investi-

(8) C. Rump, *Buchner's Repertorium für die Pharmacie*, [2] **6**, 1 (1836). (Army Medical Library, Washington.)

(9) H. Braconnot, *Ann. chim. phys.*, [3] **9**, 250 (1843).

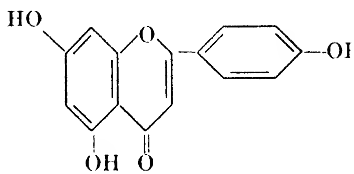
(10) A. Von Planta and W. Wallace, *Ann.*, **74**, 262 (1850).

(11) A. Lindenborn, Inaugural Dissertation, Würzburg (1867); *Chem. Centr.*, **I**, 928 (1897). See also references 12 and 13.

(12) E. Vongerichten, *Ber.*, **9**, 1121 (1876).

gation. In 1897 Perkin[13] boiled "crude apigenin" with a strong aqueous solution of potassium hydroxide and was able to establish the production of phloroglucinol and *p*-hydroxyacetophenone. After the fusion of "crude apiin" with caustic potash he identified the foregoing substances and also protocatechuic acid, the presence of which was puzzling; he suggested that it is produced through some secondary oxidation of the *p*-hydroxyacetophenone. [It has become probable from later knowledge that the protocatechuic acid came from some petroselinin in the "crude apiin."] Perkin regarded the "crude apigenin" as a single substance and suggested the formula I for it, which readily accounts for its alkaline

$C_{15}H_{10}O_5$ =



I

Perkin's formula for apigenin (1897)

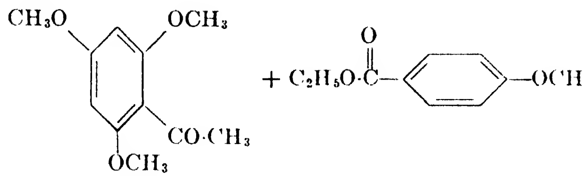
decomposition to yield phloroglucinol and *p*-hydroxyacetophenone. This inferred structure was proved rigorously later by v. Kostanecki and coworkers, as will now be described.

V. Synthesis of 5,7,4′-Trihydroxyflavone by v. Kostanecki and the Establishment of Its Identity with Natural Apigenin

The establishment of the structure of apigenin as that of 5,7,4′-trihydroxyflavone was accomplished by v. Kostanecki and coworkers[2] through the synthesis that is shown by the formulas II to VI. The synthetic trihydroxyflavone melted sharply at 347° whereas "crude apigenin" had been reported by Vongerichten[1] as subliming and partially decomposing at 292–295°; obviously the two substances were not the same. On the other hand, the 7,4′-dimethyl and diethyl ethers of the synthetic trihydroxyflavone had the melting points that had been reported by Perkin[13] for the similar derivatives of "crude apigenin." The acetates of these synthetic ethers also had properties which agreed with those that Perkin had reported for analogous derivatives of "crude apigenin." These clues led v. Kostanecki and his coworkers to repeat the acid hydrolysis of "crude apiin"; through improvement of the directions they were able to obtain from it almost pure apigenin (m. p. 343°), several derivatives of which were identical with the analogous synthetic products by direct test. Thereafter the name apigenin of the literature

(13) A. G. Perkin, *J. Chem. Soc.*, **71**, 805 (1897).

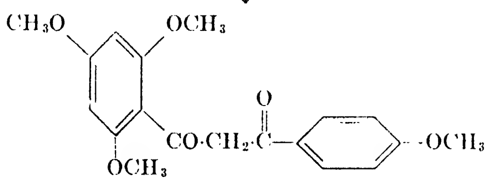
became restricted to the designation of a single chemical entity, 5,7,4′-trihydroxyflavone.



II
Phloroacetophenone trimethyl ether

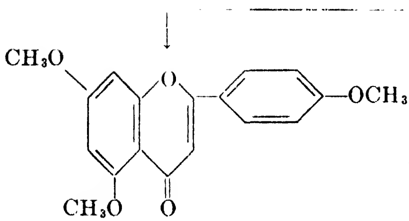
III
Ethyl anisate

(Metallic sodium)

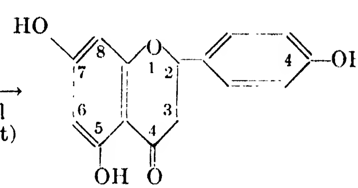


IV
2,4,6,4′-Tetramethoxybenzoylacetophenone

(HI, short treatment)



(HI, full treatment)



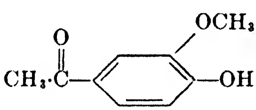
V
5,7,4′-Trimethoxyflavone

VI
5,7,4′-Trihydroxyflavone (true apigenin)

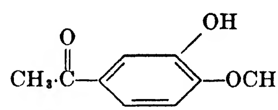
VI. Isolation of Diosmetin from "Crude Apigenin" and Its Identification as 5,7,3′-Trihydroxy-4′-methoxyflavone

It became evident from the advances in v. Kostanecki's laboratory that "crude apigenin" was a mixture of true apigenin and at least one other substance; the further investigation of "crude apigenin" was then undertaken by Vongerichten through arrangement with v. Kostanecki. Vongerichten[1] soon isolated a second aglycon from it by fractional crystallization from alcohol; it proved to be, like apigenin, a substituted flavone (m. p. 250°) which was evidently a luteolin monomethyl ether because luteolin (5,7,3′,4′-tetrahydroxyflavone) was obtained from it in

the course of the methoxyl estimation by the Zeisel procedure. Its methyl group was in the catechol portion because its treatment with boiling caustic alkali solution resulted in the production of phloroglucinol and a phenolic ketone (m. p. 94–95°). This phenolic ketone, from which protocatechuic acid (3,4-dihydroxybenzoic acid) was obtained by alkali fusion, was not acetovanillone ((VII), m. p. 115°) and was therefore assumed to be acetoisovanillone (VIII), a compound that had not been known previously. In later years Oesterle and Wander[3] prepared the same substance through the alkali fusion of diosmetin and found its m. p. to be 85–86°; they proved that it must be acetoisovanillone rather than acetovanillone because they obtained isovanillic acid from its oxidation. Vongerichten inferred from his data that the luteolin monomethyl ether which accompanies apigenin in "crude apigenin" is 5,7,3′-trihydroxy-4′-methoxyflavone. In later years Robinson and coworkers

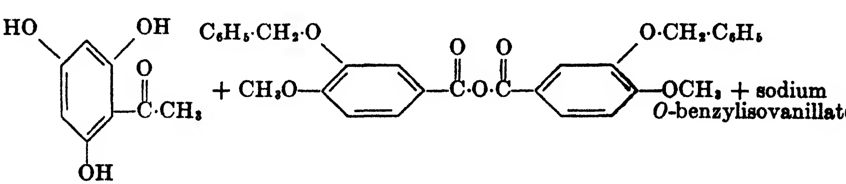


VII
Acetovanillone
(m. p. 115°)



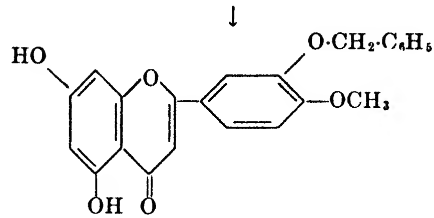
VIII
Acetoisovanillone
(m. p. 94° (V.); 86° (O. and W.))

have synthesized the substituted flavone having this structure (XIII) and also the 5,7,4′-trihydroxy-3′-methoxyflavone; the melting point of the latter was 330–331° and that of the former 253–254°. Both Vongerichten's luteolin monomethyl ether (m. p. 250°) from "crude apigenin" and the naturally occurring diosmetin (m. p. 254°) are thus 5,7,3′-trihydroxy-4′-methoxyflavone; this conclusion is confirmed by the equality of the melting points that have been reported for the triacetate (5,7,3′-triacetoxy-4′-methoxyflavone) of each of these three substances, 195° (Vongerichten[1]), 195–196° (Oesterle and Wander[3]), 195–196° (Lovecy, Robinson and Sugasawa[5]); the 5,7,4′-triacetoxy-3′-methoxyflavone melts at 220–221°. The syntheses of diosmetin and its isomer by Robinson and coworkers[5] are illustrated by formulas IX to XIII.



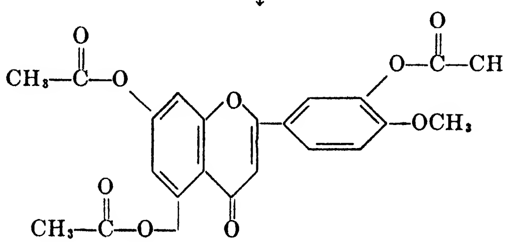
IX
Phloroacetophenone

X
O-Benzylisovanillic anhydride



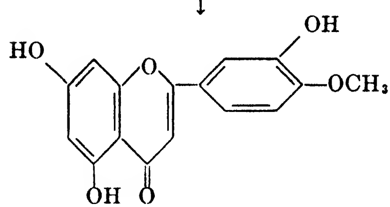
XI
3′-Benzyldiosmetin (5,7-Dihydroxy-3′-benzyloxy-4′-methoxyflavone)

(Acetic acid and HCl followed by acetic anhydride and pyridine)



XII
5,7,3′-Triacetoxy-4′-methoxyflavone

(Deacetylation by KOH)



XIII
5,7,3′-Trihydroxy-4′-methoxyflavone (Diosmetin, Luteolin 4′-methyl ether)

The synthesis of 5,7,4′-trihydroxy-3′-methoxyflavone was performed in an analogous way, substituting *O*-benzylvanillic anhydride and sodium *O*-benzylvanillate in place of the substances of the isovanillic acid series.

VII. The Carbohydrate Components of the Parsley Glycosides

Previous to 1901, the year in which Vongerichten[14] recognized the pentose apiose as a constituent of "crude apiin," it had been assumed that D-glucose was the only sugar that resulted from the acid hydrolysis of the crystalline parsley glycosides. From the percentage of "crude apigenin" that was obtained from "crude apiin" Vongerichten[12] had

(14) E. Vongerichten, *Ann.*, **318**, 121 (1901).

estimated as early as 1876 that "crude apiin" was composed of one molecule of "crude apigenin" and two molecules of D-glucose, and this inference agreed fairly well with the combustion analyses of "crude apiin." The discussion of such calculations in several of the early articles will be omitted because it became evident later that the substances were mixtures rather than pure chemical entities and that one of the two sugar components of "crude apiin" is not a hexose but rather a pentose. However, if one reinterprets today the early data of Lindenborn, Perkin and Vongerichten through the assumption that "crude apiin" consists largely of apiin, and "crude apigenin" largely of apigenin, there can be little doubt that apiin is composed of one molecule of apigenin, one of D-glucose and one of apiose. The identification of the hexose of apiin was made secure by Vongerichten[14] through the crystallization of the sugar (D-glucose). The constitution of the second glycoside, petroselinin, is not so well established as that of apiin in respect to its sugar components but there is no doubt that its aglycon is diosmetin.

1. *Vongerichten's "d-Glucoseapigenin" (7-Apigenin β-D-glucopyranoside)*

Vongerichten[14] found that "crude apiin" is hydrolyzed in two stages by mild acid treatment and that the first stage produces a reducing sugar (apiose) and a new glucoside which he named "*d*-glucoseapigenin." He crystallized this glucoside and therefore may have obtained it in pure condition. In a later article he and Müller[15] reported its $[\alpha]_D$ value as $-78°$ (solvent not stated but one may infer from the context that it was water or alcohol). The old observations furnish evidence that appears to justify classification of the glycoside at the present time as 7-apigenin β-D-glucopyranoside (XV). The glycoside is hydrolyzed readily by almond emulsin[14,15] and therefore it is a β-D-glucopyranoside because neither furanosides nor α-D-glucopyranosides are hydrolyzed by this mixture of enzymes. The same conclusions regarding both the pyranoid structure and the β-anomeric configuration are reached independently from the observation of Vongerichten and Müller[15] that "*d*-glucoseapigenin" is converted to levoglucosan (now known to be D-glucosan $<1,5>\beta<1,6>$) by heating with strong aqueous sodium hydroxide solution; this behavior, first observed by C. Tanret[16] in the case of natural picein (*p*-hydroxyacetophenone β-D-glucopyranoside), is typical of aryl β-D-glucopyranosides. Thus phenyl β-D-glucopyranoside is readily transformed by this reaction to phenol and levoglucosan whereas phenyl α-D-glucopyranoside is not affected.[17]

(15) E. Vongerichten and F. Müller, *Ber.*, **39**, 241 (1906).

(16) C. Tanret, *Bull. soc. chim.*, [3] **11**, 949 (1894); *Compt. rend.*, **119**, 158 (1894).

(17) Edna Montgomery, N. K. Richtmyer and C. S. Hudson, *J. Am. Chem. Soc.*, **65**, 3 (1943).

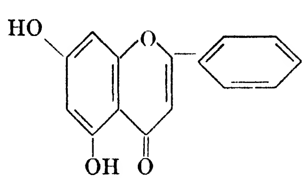
The strongly negative rotation of "*d*-glucoseapigenin" is compatible only with the preceding conclusion that it is a beta rather than an α-D-glucoside.

Since apigenin (VI) carries phenolic groups at positions 5,7 and 4′, it is necessary to know which one of these is joined with the glucose moiety in "*d*-glucoseapigenin"; decision of this question was attained in one of the early researches of Vongerichten, as will now be explained.

2. *Vongerichten's 4′-Methyl Ether of "Crude Apiin"*

Vongerichten's[1,14] methylation of "crude apiin" by refluxing its solution in aqueous potassium hydroxide with methyl iodide yielded a monomethyl ether from which a monomethyl ether of "crude apigenin" was obtained through acid hydrolysis. This monomethyl ether of "crude apigenin" yielded phloroglucinol and *p*-methoxyacetophenone as scission products from the action of aqueous alkali and therefore it was essentially apigenin 4′-methyl ether. Accordingly, only positions 5 and 7 of the phloroglucinol moiety of apigenin remained as possibilities for the attachment of the carbohydrate portions in apiin. Vongerichten concluded through the following quoted (in translation) evidence,[14] that the hydroxyl group on carbon atom 5 is free in apiin and consequently free in "*d*-glucoseapigenin."

"Monomethylapiin forms with sodium hydroxide a deeply yellow insoluble sodium salt; therefore one hydroxyl group of the phloroglucinol moiety has not been methylated. The question of the position of this hydroxyl group is readily answered through the results of the researches of v. Kostanecki and his students on xanthone and flavone derivatives. Dreher and v. Kostanecki[18] found that the hydroxyl group which was in ortho position to the carbonyl group of the xanthones was difficult to methylate under usual conditions. In the case of chrysin (XIV), the structure of which is closely similar to that of apigenin, v. Kostanecki[19] obtained only a mono-



XIV
Chrysin (5,7-dihydroxyflavone)

methyl ether and this substance formed a deeply yellow sodium salt. According to v. Kostanecki 'this formation of a deeply yellow sodium salt by the chrysin methyl ether is quite analogous to the behavior of the hydroxyxanthones through the free

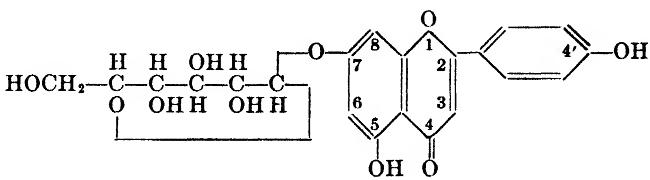
(18) E. Dreher and S. v. Kostanecki, *Ber.*, **26**, 71 (1893).

(19) S. v. Kostanecki, *Ber.*, **26**, 2901 (1893); S. v. Kostanecki and J. Tambor, *Ber.*, **28**, 2303 (1895); see also Hans Rupe's "Die Chemie der natürlichen Farbstoffe," page 19 (1900).

hydroxyl group being in ortho position to the carbonyl group.' The conclusion is therefore justified that in apiin, which belongs in the flavone group, a free hydroxyl group in ortho position to the carbonyl group resists methylation and is responsible for the formation of a deeply yellow sodium salt; accordingly, this hydroxyl group in apiin is not united with a sugar component."

In this way Vongerichten restricted the carbohydrate (D-glucosyl) group to position 7 of apigenin; it also followed that the apiose moiety is joined to the D-glucose component in a disaccharide union.

The configurational formula for Vongerichten's "*d*-glucoseapigenin" is to be written at the present time as XV. The full configurational



XV
7-Apigenin β-D-glucopyranoside
(Vongerichten's "*d*-Glucoseapigenin")

formula XV is applicable in the eventual establishment of the full formula for apiin; in apiin the apiose molecule is attached in some way to the D-glucose portion of XV.

3. *Vongerichten's Discovery of Apiose and Proof of Its Structure*

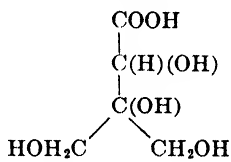
a. Apiose Phenylosazone and Apiose p-Bromophenylosazone. It has been mentioned that Vongerichten accomplished the single-stage hydrolysis of "crude apiin" to a reducing sugar and a non-reducing glycoside which he crystallized and named "*d*-glucoseapigenin." He prepared from the sugar solution a crystalline phenylosazone[20] (m. p. 155–157°) and *p*-bromophenylosazone (m. p. 211–212°); analyses of the osazones showed that the sugar is a pentose. Since it failed to yield furfural by acid treatment it appeared to be a new kind of pentose and he gave it the name apiose, of obvious derivation. In his first article concerning apiose he mentioned the possibility that it might be a ketopentose since such a structure would not be expected to yield furfural; however, in the next article[21] he was able to prove through oxidation that apiose is indeed an aldopentose but that it differs from the normal pentoses such as xylose and arabinose in possessing a branched-chain structure.

b. Apionic Acid, Its Phenylhydrazide and Its Calcium and Strontium Salts. The oxidation[21] of a crude apiose solution with bromine water

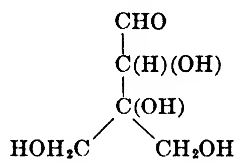
(20) (*a*) Reference 14; (*b*) E. Vongerichten and F. Müller, *Ber.*, **39**, 235 (1906).
(21) E. Vongerichten, *Ann.*, **321**, 71 (1902).

generated a pentonic acid which was amorphous but it was characterized through a crystalline phenylhydrazide (prisms, m. p. 126–127°), a crystalline strontium salt and an amorphous calcium salt. Analyses of these showed that apionic acid is a pentonic acid; therefore apiose must be an aldopentose.

c. Reduction of Apionic Acid to Isovaleric Acid. The reduction of the acid constituent of "amorphous calcium apionate" by hydriodic acid and phosphorus yielded a volatile acid; from his tests of its properties Vongerichten concluded that it was isovaleric acid. According to present standards his tests for characterization were not as precise as could be desired; however, Schmidt[22] established subsequently in a precise manner that Vongerichten's conclusion is correct. Since isovaleric acid has the structure $(CH_3)_2 \cdot CH \cdot CH_2 \cdot COOH$, Vongerichten proposed the structural formulas XVI and XVII for apionic acid and apiose, respectively, and he designated the branched-chain pentose systematically as a "β-hydroxymethylerythrose." Its formula indicates of course that it can be regarded equally well as a "β-hydroxymethylthreose."



XVI



XVII

Vongerichten's formulas for apionic acid and apiose

The configuration of the single asymmetric carbon atom in this formula was unknown. The customary formulas of apiose osazones would contain no asymmetric carbon atom; Vongerichten and Müller[20b] could detect no optical activity of the two osazones which they prepared.

d. Apiose α-Benzyl-α-phenylhydrazone. Vongerichten and Müller[20b] made the important discovery of the first crystalline hydrazone of apiose, its α-benzyl-α-phenylhydrazone (m. p. 135°), a substance from which apiose in nearly pure though amorphous condition may be prepared. They reported for the regenerated apiose the $[\alpha]^{20}_{D}$ value of +3.8° in aqueous solution.

VIII. The Configuration of Apiose

1. *Schmidt's Reduction of Authentic Apionic Acid to Isovaleric Acid*

Twenty-four years elapsed after the conclusion of Vongerichten's researches on apiose before the study of its structure and configuration was undertaken by Schmidt.[22] He prepared 500 grams of "purified

(22) O. T. Schmidt, *Ann.*, **483**, 115 (1930).

apiin" from 200 kilograms of fresh parsley herb by Vongerichten's procedure;[12] this yield of 2.5 g. per kilo and Vongerichten and Müller's record[20b] of 3.75 g. per kilo give an idea of the approximate amount of mixed crystalline glycosides that an investigator may expect to obtain from the herb. The writer has found no record of the yield from parsley seed. Schmidt describes (in translation) his "purified apiin" as follows.

"The material consisted of light yellow crystals (pointed prisms) the several samples of which melted between 225° and 234°. It was of very low solubility in cold water or alcohol; although the gelatinous form of the glycosides dissolves considerably in either of these solvents on heating, the crystalline form is difficultly soluble in them but it does dissolve in boiling 70% alcohol. Pyridine dissolves it in the cold rather readily and the addition of dry ether then causes crystallization. The methoxyl content of the preparation was 1%, which is interpreted as showing the presence of about 20% of hydroxyapiin methyl ether [petroselinin], of five% methoxyl content."

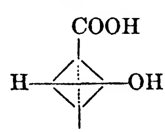
Schmidt's mixed glycoside material seems therefore to have consisted essentially of four parts of true apiin and one part of petroselinin. Since his continuation of the old practice of applying the name "apiin" to such mixtures may confuse the reader of his article, the use of the word has been avoided in the translated quotation. The selective hydrolysis of the mixture of glycosides by dilute sulfuric acid and the separation of a sugary sirup from the other products were performed according to the directions of Vongerichten.[21] The fermentable sugar, which amounted to about ten percent of the total sugars and presumably consisted of D-glucose, was removed by the action of yeast. There was thus obtained about 7.5 g. of apiose in an absolute alcohol solution as the yield of apiose from 30 g. of the mixed glycosides; the apiose was then converted to the crystalline α-benzyl-α-phenylhydrazone (10.5 g.), m. p. 136°. Recrystallization from chloroform gave the pure substance of m. p. 137–138°; its rotation in pyridine solution is strongly to the left and the values for two wave lengths (the mercury lines 546 and 579) were $[\alpha]$ −94.0° and −78.5° respectively, the concentration being about 5% and the temperature 20–25°. It is readily soluble in cold pyridine and in hot absolute alcohol but of low solubility in chloroform, ethyl acetate, benzene, ether, ligroin and carbon tetrachloride. It was free of methoxyl content and it showed the theoretical percentage of nitrogen. Apiose in sirupy form was regenerated from this pure hydrazone by the formaldehyde procedure; the light yellow sirup was dried to constant weight in a vacuum desiccator and its $[\alpha]^{15}_{D}$ value found to be +5.6° (water, conc. about 10%), a value near that which Vongerichten and Müller[20b] had reported (+3.8°) for amorphous apiose which had been regenerated from the same hydrazone. Schmidt oxidized this authentic apiose to apionic acid with

barium hypoiodite according to the procedure of Goebel[23] and succeeded in obtaining calcium apionate in pure crystalline form as prisms containing two molecules of water of crystallization. When the acid is liberated from an aqueous solution of the potassium salt by the addition of hydrochloric acid its initial $[\alpha]_{546}$ value was $-20°$; there followed a mutarotation to the end value of $-34.6°$, due presumably to lactone formation. Vongerichten's calcium apionate was amorphous and it had been prepared from sugary material that had not been purified through any crystalline derivative of apiose; it was not therefore certain that the volatile acid which he had obtained in small yield by the reduction of such "calcium apionate" with hydriodic acid and phosphorus had come from apionic acid. Accordingly, Schmidt repeated the reduction, using pure crystalline calcium apionate; the yield of purified volatile acid was only 4.5% of theory but the substance was conclusively identified as isovaleric acid (isopropylacetic acid, $(CH_3)_2{\cdot}CH{\cdot}CH_2{\cdot}COOH$) through the preparation of its *p*-bromophenacyl ester[24] (m. p. 68°). Schmidt's careful investigation has thus established conclusively the original structural formulas of Vongerichten for apionic acid (XVI) and apiose (XVII).

2. *Schmidt's Evidence for the Configuration of Apionic Acid and Apiose*

During the interval between Vongerichten's and Schmidt's investigations, there were discovered several empirical rules through which the configuration of the α-carbon atom of many α-hydroxycarboxylic acids may be inferred. Schmidt applied three of these generalizations, the salt-acid rule[25] and the phenylhydrazide and amide rules,[26] to the appropriate derivatives of apionic acid; Table I shows the comparisons which he published.

Levene's acid-salt rule indicates that the configuration of the α-carbon atom in all these acids is the same, namely, the grouping XVIII of



XVIII

D-lactic or D-glyceric acid. The dextrorotation of the three phenylhydrazides and of the amide of lactic acid leads to the same conclusion

(23) W. F. Goebel, *J. Biol. Chem.*, **72**, 809 (1927).

(24) W. L. Judefind and E. E. Reid, *J. Am. Chem. Soc.*, **42**, 1043 (1920).

(25) P. A. Levene, *J. Biol. Chem.*, **23**, 145 (1915); P. A. Levene and G. M. Meyer, *ibid.*, **26**, 355 (1916).

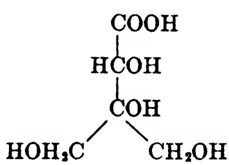
(26) P. A. Levene, *J. Biol. Chem.*, **23**, 145 (1915); C. S. Hudson, *J. Am. Chem. Soc.*, **39**, 462 (1917); **40**, 813 (1918).

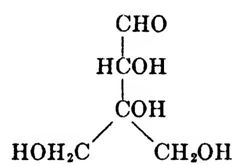
TABLE I
Comparisons of Rotations in the Series of α-Hydroxycarboxylic Acids

Acid	*Free acid* [M]D	*Sodium salt* [M]D	*Phenylhydrazide* [M]D
D-Lactic acid	−2.2 to 3.6°	+12.2°	+ 19.6° (Amide)
D-Hexahydromandelic acid	−42°	+13.1°	+134°
D-Gluconic acid	0.0°	+25.7°	+ 51.5°
Apionic acid[a]	−33.2°	− 2.45°	+ 76.8°

[a] The values in this line refer to the yellow-green line (546) of mercury vapor; the rotations would be expected to be about 15% less for the sodium D-line (589), but this change would not influence the argument.

through the phenylhydrazide and amide rules. This evidence from rotatory relations is particularly strong because the α-carbon atom of apionic acid is the only asymmetric carbon atom in its structure. On this evidence it is now believed that the configurational formula of apionic acid is XIX and of apiose XX, as respects the aldehydo type of this pentose.


XIX


XX

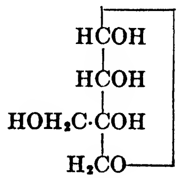
Schmidt's configurational formulas of apionic acid and apiose

IX. Discussion of Possible Formulas for Apiin and Petroselinin

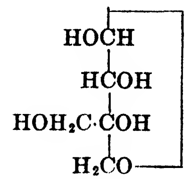
It has been shown on page 68 that the formula for apiin should express the elements of structure that are present in formula XV for 7-apigenin β-D-glucopyranoside and that the apiose moiety is attached in some unknown way to the D-glucopyranosyl component. If an acetal structure be regarded as improbable, an apiofuranosyl arrangement is probably present since an apiopyranosyl structure is not possible for this branched-chain pentose. Theory indicates that four apiofuranosyl structures come into consideration; they are the α and β types from two furanoses, as shown by the named formulas XXI to XXIV, all of which are derivable theoretically from the aldehyde formula of apiose (XX).[27] In this connection it will be observed that carbon atom 3 of apionic acid (XIX) also becomes asymmetric when the acid generates γ-lactones and that in consequence two γ-lactones of apionic acid are to be expected.

(27) C. S. Hudson, *Advances in Carbohydrate Chemistry*, **3**, 22 (1948).

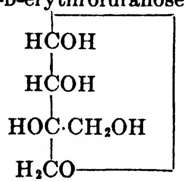
Apiin can be described at present as very probably a 7-apigenin apio-



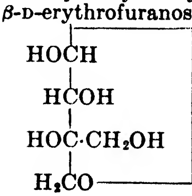
XXI
3-*C*-Hydroxymethyl-
α-D-erythrofuranose



XXII
3-*C*-Hydroxymethyl-
β-D-erythrofuranose



XXIII
3-*C*-Hydroxymethyl-
β-L-threofuranose



XXIV
3-*C*-Hydroxymethyl-
α-L-threofuranose

furanosyl-β-D-glucopyranoside; this designation leaves for later determination the specification of the structure of the apiofuranosyl radical of apiin and the place of its attachment to the D-glucosyl structure. The writer suggests the trivial name *apiinibiose* for the disaccharide of apiin. Evidently its disaccharide union cannot be at position 5 of D-glucose. The ease with which apiin is hydrolyzed by acids to apiose and 7-apigenin β-D-glucopyranoside agrees with the view that apiin contains a furanosyl form of apiose; on the other hand this ease of hydrolysis makes the isolation of the disaccharide a most difficult problem which has so far received no solution. Possibly there may be found some enzyme which will hydrolyze apiin to apigenin and apiinibiose, even though Vongerichten reported[14] that apiin is not split by emulsin.

The evidence for any formula for petroselinin is very indefinite beyond the fact that the aglycon is diosmetin. Analytical values for samples of "crude apiin" that contained much petroselinin suggest that the carbohydrate component of petroselinin is also a disaccharide and the inference has been made by Vongerichten that it is composed of apiose and D-glucose; but such matters are speculation at present because petroselinin has not yet been obtained in even approximately pure condition.

X. The Action of Alkali on Apiin and 7-Apigenin β-D-Glucopyranoside

The conclusion of Vongerichten[14] that the apigenin aglycon of "crude apiin" is attacked by boiling sodium hydroxide solution (25%, 5 hours)

without any change of the carbohydrate portion is very worthy of note. He isolated nearly a quantitative amount of *p*-hydroxyacetophenone from the reaction mixture and he believed that the other product was a phloroglucinol glycoside of the biose that is here termed apiinibiose. This product, which he named "apioseglucosephloroglucin," was amorphous and no later investigators have studied it. When Vongerichten and Müller[15] subjected their "*d*-glucoseapigenin" (7-apigenin β-D-glucopyranoside, see p. 68) to similar alkaline treatment they obtained some levoglucosan, which was accurately identified, and an amorphous substance which they considered to be a D-glucoside of phloroglucinol, and named it "*d*-glucosephloroglucin"; its $[\alpha]_D$ value in water was $-25°$ and they reported that it was not hydrolyzed by almond emulsin. Subsequently Fischer and Strauss[28] synthesized pure crystalline phloroglucinol β-D-glucoside, found its $[\alpha]_D$ value in water to be $-75°$ and noted that it is readily hydrolyzed by emulsin; it proved to be identical with the glycoside phlorin which Cremer and Seuffert[29] had produced through the action of barium hydroxide on phloridzin. It would seem that Vongerichten's amorphous "apioseglucosephloroglucin" and "*d*-glucosephloroglucin" were decidedly impure products; nevertheless the records concerning them are suggestive that his discovery of the alkaline degradations of the parsley glycosides may become of considerable importance in years to come, especially in relation to future studies of the carbohydrate components of these glycosides.

(28) E. Fischer and H. Strauss, *Ber.*, **45**, 2467 (1912).

(29) M. Cremer, *Münch. med. Wochschr.*, **58**, 1713 (1911); M. Cremer and R. W. Seuffert, *Ber.*, **45**, 2565 (1912).

BIOCHEMICAL REDUCTIONS AT THE EXPENSE OF SUGARS

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Contents

I. Introduction

In the course of biological assimilation, carbohydrates are produced by the reduction of carbon dioxide; however, the character of such natural photosynthesis has not been elucidated. In other biochemical processes carbohydrates act as reducing agents; somewhat greater insight into processes of this type has been gained. The development of this knowledge is closely connected with the advances in our understanding of the character of alcoholic fermentation, a natural process

which includes reduction phases. Since inanimate nature does not provide free hydrogen, hydrogen is created during the metabolism of living beings by what may be termed, broadly, an oxidation-reduction process.

The end product of alcoholic sugar cleavage, ethyl alcohol, is in the last resort formed by a dismutation between acetaldehyde and 3-phospho-D-glyceraldehyde, the latter being oxidized to 3-phospho-D-glyceric acid during the process. In an analogous manner L-lactic acid is formed in the animal organism by the dismutation of carboxylated acetaldehyde, namely pyruvic acid, and 3-phospho-D-glyceraldehyde. A detailed presentation of the glycolytic processes[1] can be found in the book by Sumner and Somers and an abbreviated one in an article by Neuberg.

After pyruvic acid and acetaldehyde had been identified as intermediate products of glycolysis, sugar degradation could be formulated schematically by the following equations:

$$C_6H_{12}O_6 \rightarrow 2\ CH_3{\cdot}CO{\cdot}COOH + 4\ H \qquad (I)$$
$$C_6H_{12}O_6 \rightarrow 2\ CH_3{\cdot}CHO + 2\ CO_2 + 4\ H \qquad (II)$$

Normally the four hydrogen atoms are required for the final reduction of acetaldehyde and of pyruvic acid. The hydrogen does not appear in the free state but is reversibly bound to a hydrogen acceptor. The term "potentieller oder labiler Gärungswasserstoff" (potential or labile hydrogen of fermentation) has been applied provisionally to this hydrogen. Hydrogen in this state manifests itself when, during a fermentation, yeast reduces an added substance. Obviously the hydrogen is thereby withdrawn from its normal acceptors, which are acetaldehyde and pyruvic acid. Such "phytochemical reductions" have been carried out in large numbers. (The term "phytochemical reduction" first appeared in 1914.[2])

A reducing action of fermenting yeast was first observed many decades ago. In 1874 Dumas[3] reported that on the addition of finely powdered sulfur to a suspension of fresh brewer's yeast in a sugar solution, hydrogen sulfide is liberated; he concluded that "la levure ou ses produits agissent donc comme hydrogènants." According to Rubner,[4] the hydrogen sulfide is admixed with a mercaptan, probably ethyl mercaptan. This mercaptan formation, appearing quite possible nowadays (see p. 93), could not be confirmed in yeast maceration juice by

(1) J. B. Sumner and G. F. Somers, "Chemistry and Methods of Enzymes," Academic Press, New York, 2nd ed., pp. 343 and 346 (1947). C. Neuberg, *Am. Brewer*, **75**, 22 (May, 1942).

(2) C. Neuberg and E. Welde, *Biochem. Z.*, **60**, 472 (1914).

(3) J. B. Dumas, *Ann. chim. phys.*, [5] **3**, 92 (1874).

(4) M. Rubner, *Arch. Hyg.*, **19**, 187 (1893).

Hahn.[5] Reasoning from the observation that proteins and other substances which contain sulfhydryl groups produce hydrogen sulfide on addition of sulfur, Heffter[6] denied that a biochemical reduction process took place at all. As a generalization this negation seems definitely unjustified (see pp. 93 and 95).

As far back as 1912 Neuberg and Kerb[7] observed that during the fermentation of pyruvic acid some of the primarily formed acetaldehyde is transformed to ethyl alcohol. The only fact known at that time concerned the behavior of furfural; in a communication fallen into oblivion since, Windisch[8] in 1898 described the disappearance of furfural under the anaerobic conditions of alcoholic fermentation by means of living yeast. The occurrence of furfuryl alcohol during this process was shown thirteen years later by Lintner and von Liebig.[9] They obtained a yield of 10%; no corresponding product of oxidation was found among the other compounds formed (*cf.* p. 82). Kostytschew and Huebbenet,[10] and also Lebedew and Griaznoff,[11] showed soon afterwards that not only acetaldehyde in the nascent state (as produced from pyruvic acid by carboxylase) but also added acetaldehyde can be converted to ethanol by yeast. In a series of investigations Neuberg and his collaborators experimentally proved that the homologs of acetaldehyde are hydrogenated by a true reduction process and that this hydrogenation is not confined to some few aldehydes, but that fermenting yeast quite generally exerts a reducing action on added substances of many totally different types. This new biochemical process, which is particularly useful in the preparation of various complicated substances, is known as "phytochemical reduction." (Possibly the more general term "bioreduction" would be preferable, since animal cells also act in an analogous way.)

II. The Phytochemical Reduction of Aldehydes

1. *Phytochemical Reduction of Simple Aliphatic Aldehydes*

Acetaldehyde itself is transformed by entirely different mechanisms, partly by acyloin condensation, as was found in 1921,[12] and partly by

(5) M. Hahn, *Maly's Jahresber. Tierchemie*, **29**, 935 (1900).

(6) A. Heffter, *Mediz.-Naturwiss. Archiv*, **1**, 81 (1907); *Chem. Centr.*, II, 822 (1907); *Arch. exptl. Path. Pharmakol., Festschrift für Schmiedeberg*, 253 (1908).

(7) C. Neuberg and J. Kerb, *Z. Gärungsphysiol.*, **1**, 114 (1912).

(8) W. Windisch, *Wochschr. Brau.*, **15**, 189 (1898); *Chem. Centr.* I, 1214 (1898).

(9) C. J. Lintner and H. J. von Liebig, *Z. physiol. Chem.*, **72**, 449 (1911).

(10) S. Kostytschew and E. Huebbenet, *Z. physiol. Chem.*, **79**, 359 (1912).

(11) A. Lebedew and N. Griaznoff, *Ber.*, **45**, 3263 (1912).

(12) C. Neuberg and J. Hirsch, *Biochem. Z.*, **115**, 282 (1921); C. Neuberg and E. Reinfurth, *ibid.*, **143**, 553 (1923).

oxido-reduction as predicted by Bach.[13] Many simple aliphatic aldehydes have been hydrogenated phytochemically. These include commerically available valeraldehyde (a mixture of isovaleraldehyde and optically active methylethylacetaldehyde),[14] butyraldehyde,[15] *n*-valeraldehyde,[16] *n*-heptanal (oenanthaldehyde),[15] *n*-caproaldehyde[17] and isocaproaldehyde.[18]

Valeraldehyde is of special interest because of its connection with fusel oil and its natural occurrence in ethereal oils and in leaves. Phytoreduction of valeraldehyde has been accomplished in the following manner. The starting material was a fraction of commercial valeraldehyde (mixture of isomers) prepared from fermentation amyl alcohol (fusel oil) and boiling between 91° and 93°. The valeraldehyde was either added dropwise to the fermenting mixture of sucrose and yeast or it was used in the form of its ammonia compound, namely as a mixture of the aldehyde with an equivalent amount of ammonia. The time required for dropwise addition varied between 40 and 75 minutes. Digestion of the mixtures in all cases was carried out at room temperature. To recover the reaction products the fermented mixture was distilled and fractionated. The amyl alcohol that was formed was isolated by ether extraction of the distillate which previously had been enriched, and sometimes saturated with potassium carbonate. The dry ether extracts were then fractionated in the "birectificator" (birectifier), a very useful apparatus described by Heinzelmann.[19] The best yield of amyl alcohol was 84.1%; a poor yield was 66.4%. Control experiments showed that sugar and yeast alone in the amounts used gave no more than traces of amyl alcohol. In conformity with the composition of the starting material, the resulting amyl alcohol had a boiling point of 127°–131° and consisted of a mixture of isobutylcarbinol (2-methyl-4-butanol) and *sec.*-butylcarbinol (2-methyl-1-butanol). Polarimetric tests showed that the ratio of the two alcohols in the phytochemical reduction product corresponded with that of the isomeric constituents of the valeraldehyde. The best yields were obtained from valeraldehyde in the presence of ammonia.

There are two possible mechanisms for forming amyl alcohol from valeraldehyde. It may result from a dismutation, yielding valeric acid and amyl alcohol in equivalent amounts, or it may result from direct hydrogenation of the aldehyde. The yields that are obtained are clear

(13) A. Bach, *Biochem. Centr.*, **9**, 10 (1909).
(14) C. Neuberg and H. Steenbock, *Biochem. Z.*, **52**, 494 (1913).
(15) K. Ohta, *Biochem. Z.*, **59**, 183 (1914).
(16) C. Neuberg and F. F. Nord, *Biochem. Z.*, **62**, 482 (1914).
(17) C. Neuberg and F. F. Nord, *Biochem. Z.*, **67**, 24 (1914).
(18) C. Neuberg and N. N. Iwanoff, unpublished.
(19) G. Heinzelmann, *Z. Spiritusind.*, **35**, 612 (1912).

evidence that in the main the second reaction is taking place; any reaction of the Cannizzaro type could not produce more than 51.2% of amyl alcohol at most.

According to Ehrlich[20] the formation of fusel oil alcohols from nitrogen-containing materials in the mash can take place only in the presence of fermentable sugar and only with living yeast. Buchner and Meisenheimer,[21] and also Pringsheim,[22] could not recognize the process as resulting from other than *living* yeast. In connection with this question it is important to know whether the alcohols of fusel oil can be formed from their respective aldehydes by fermentation in the absence of complete yeast cells. To elucidate this point Neuberg and Steenbock[23] set up the requisite experiments with cell-free "yeast juice." It was ascertained that, in the presence of sugar, such active zymase extracts are able to reduce valeraldehyde to amyl alcohol. *The reaction was thus shown to be enzymatic.* This is the first case where a purely enzymatic reduction of a product of intermediary metabolism at the expense of sugar was demonstrated (see also p. 95).

2. *Phytochemical Reduction of Unsaturated Aliphatic Aldehydes*

The study of phytochemical reduction was extended[24] to the important group of olefinic terpene derivatives. As an example of the investigations in this series, dextrorotatory citronellal may be cited; it was reduced by the usual experimental methods. In spite of the slight solubility of citronellal in water, reduction to the corresponding terpene alcohol, dextrorotatory citronellol, could be accomplished in more than 60% yield after a period of bottom yeast action that was somewhat longer than is necessary for valeraldehyde. In the course of the reaction the starting material disappeared completely; apparently the part not reduced is transformed in some complicated way. Again the degree of reduction is greater than could be accounted for by a dismutation reaction. The reduction of citral to geraniol takes an analogous course.[25]

3. *Phytochemical Reduction of Aromatic and Aliphatic–Aromatic Aldehydes*

In this class, benzaldehyde has been converted to benzyl alcohol,[26] salicylaldehyde to saligenin,[27] cinnamaldehyde to cinnamyl alcohol[28] and

(20) F. Ehrlich, *Biochem. Z.*, **2**, 52 (1906); **36**, 496 (1911).
(21) E. Buchner and J. Meisenheimer, *Ber.*, **39**, 3208 (1906); **43**, 1774 (1910).
(22) H. Pringsheim, *Ber.*, **39**, 3713 (1906).
(23) C. Neuberg and H. Steenbock, *Biochem. Z.*, **59**, 188 (1914).
(24) P. Mayer and C. Neuberg, *Biochem. Z.*, **71**, 174 (1915).
(25) C. Neuberg and E. Kerb-Etzdorf, *Biochem. Z.*, **92**, 111 (1918).
(26) C. Neuberg and E. Welde, *Biochem. Z.*, **62**, 477 (1914).
(27) P. Mayer, *Biochem. Z.*, **62**, 459 (1914).
(28) Elisabeth Róna, *Biochem. Z.*, **67**, 137 (1914).

o-nitrobenzaldehyde to *o*-nitrobenzyl alcohol.[29] Special precautions were necessary to convert phenylacetaldehyde to phenylethyl alcohol[26] because the aldehyde decomposed in the fermentation mixture to give a thick yellow precipitate. This is probably due to the acidity of the yeast suspension, since phenylacetaldehyde is very sensitive to acids. The aldehyde was therefore combined with the calculated amount of ammonia.[30] Since the yield depends to a large extent on the *p*H of the fermentation mixture, the addition of calcium carbonate increased the yield of the phenylethyl alcohol. In the case of tetraacetyl-gluco-coniferyl aldehyde the phytochemical reduction is so mild that tetra-acetyl-gluco-coniferyl alcohol forms without splitting off the acetyl groups.[31] Coniferyl aldehyde itself and methoxymethylconiferyl aldehyde yield the corresponding coniferyl alcohols.[32] In both cases the double bonds (see pp. 79, 82 and 91) remain intact.

4. *Phytochemical Reduction of Racemic Aldehydes*

The results that have been described so far do not establish whether the phytochemical reduction is a biological process of the first or of the second order, that is, whether it is effected directly by a biological agent or only through catalysis by a constituent of yeast of a purely chemical transformation. Extension of the reaction to racemic aldehydes should give an insight into the mechanism of the transformation, since its dependence on physiological factors would show itself in an asymmetric course of the hydrogenation. The racemic valeraldehyde *d,l*-methyl-ethylacetaldehyde[33] was used, the experimental procedure being the same as that applied to "isovaleraldehyde" (see p. 78). Biohydrogenation of 17.2 g. of *d,l*-valeraldehyde yielded 12.4 g. (70%) of an amyl alcohol containing 16% excess of a levorotatory component. The phytochemical reduction can thus be carried out asymmetrically, a fact which establishes that it is a fermentative process. (See also pp. 79 and 95.)

5. *Phytochemical Reduction of Hydroxy Aldehydes*

D,L-Lactaldehyde, $CH_3 \cdot CHOH \cdot CHO$, is reduced by fermenting yeast to 1,2-propylene glycol. In this reaction 1,2-propylene glycol containing excess of the dextrorotatory component was obtained by Neuberg and Vercellone[34] by means of top yeast. On the other hand, acetol (see p. 84) and methylglyoxal (see p. 85) yield a levorotatory 1,2-propylene

(29) F. F. Nord, *Biochem. Z.*, **103**, 315 (1920).
(30) Consult references 14, 94 and 95.
(31) H. Pauly and K. Feuerstein, *Ber.*, **60**, 1032 (1927).
(32) H. Pauly and K. Feuerstein, *Ber.*, **62**, 305 (1929).
(33) C. Neuberg and M. Ringer, *Biochem. Z.*, **90**, 388 (1918).
(34) C. Neuberg and A. Vercellone, *Biochem. Z.*, **279**, 140 (1935).

glycol. With a Japanese bottom yeast Ochiai and Miyaki[35] obtained a propylene glycol which did not show optical activity. Even earlier, Neuberg and Kerb-Etzdorf[36] applied the method to an important hydroxy aldehyde and showed that *d,l*-acetaldol, $CH_3 \cdot CHOH \cdot CH_2 \cdot CHO$, can be smoothly converted to the corresponding alcohol, 1,3-butylene glycol; this hydrogenation is effected asymmetrically with a yield of 63.5%. The resulting glycol has a rotation of $[\alpha]_D = 10.9°$. This asymmetric hydrogenation has also been observed by Levene, Walti and Haller.[37] In the course of their investigations of the configurational relationships of carbinols, Levene and Walti[38] extended the method of phytochemical reduction to *d,l*-α-hydroxypentanal and *d,l*-α-hydroxyhexanal, thereby preparing dextrorotatory 1,2-pentanediol and 1,2-hexanediol.

6. *Phytochemical Reduction of Halogenated Aldehydes* (*and Ketones*)

The possibility of hydrogenating halogenated aldehydes and ketones by means of phytochemical reduction was tested as early as 1913; the successful results in this field clearly demonstrate the importance of this method. Lintner and Lüers[39] found that chloral hydrate can be converted to trichloroethyl alcohol. This transformation takes place so easily that, according to Willstätter and Duisberg,[40] it can be used under favorable experimental conditions as a convenient method for the preparation of the halogenated alcohol. The tribromoethyl alcohol may be prepared in an analogous manner.

Similarly, methyl α-chloroethyl ketone, α,α-dichloroacetone and α,α,β-trichlorobutyraldehyde have been converted phytochemically and in good yield to the corresponding primary or secondary halogenated alcohols.[41] Since all these alcohols show optical rotation, it seems established that the phytochemical reduction generally takes an asymmetric course. (See pp. 80, 81, 88 and 92.)

Higher plants also effect such reductions. *Nicotiana Tabacum* accumulates in its leaves considerable amounts of the β-D-glucoside and β-gentiobioside of 2,2,2-trichloroethanol if the plants are cultivated in a nutrient medium containing chloral hydrate.[42]

(35) E. Ochiai and K. Miyaki, *Biochem. Z.*, **282**, 293 (1935).
(36) C. Neuberg and E. Kerb-Etzdorf, *Biochem. Z.*, **92**, 96 (1918).
(37) P. A. Levene, A. Walti and H. L. Haller, *J. Biol. Chem.*, **71**, 466 (1927).
(38) P. A. Levene and A. Walti, *J. Biol. Chem.*, **94**, 361 (1913).
(39) C. J. Lintner and H. Lüers, *Z. physiol. Chem.*, **88**, 122 (1913).
(40) R. Willstätter and W. Duisberg, *Ber.*, **56**, 2283 (1923).
(41) P. Santomauro, *Biochem. Z.*, **151**, 48 (1924); H. K. Sen, *ibid.*, **151**, 51 (1924); L. Rosenfeld, *ibid.*, **156**, 54 (1925).
(42) L. P. Miller, *Contrib. Boyce Thompson Inst.*, **12**, 465 (1942); **13**, 185 (1943).

III. The Phytochemical Reduction of Ketones

Biological hydrogenations are of special interest in the case of processes taking place in living organisms, since the required hydrogen is not available in free form in nature and must be obtained by indirect means. These processes thus differ from oxidations, where the oxygen is readily supplied from the surrounding atmosphere, and also from dismutations. Insight into the problems of phytochemical reduction was enhanced by a study of ketones.

1. *Conversion of Methylheptenone*

The first suitable representative of this class of substances, 6-methyl-5-heptene-2-one, was investigated by Neuberg and Lewite.[43] This ketone, which is a natural constituent of ethereal oils, can also be obtained by degradation of various aliphatic and cyclic terpenes. By means of fermenting yeast it is converted to 6-methyl-5-heptene-2-ol,

$$(CH_3)_2{\cdot}C{=}CH{\cdot}CH_2{\cdot}CH_2{\cdot}CO{\cdot}CH_3 \rightarrow (CH_3)_2{\cdot}C{=}CH{\cdot}CH_2{\cdot}CH_2{\cdot}CHOH{\cdot}CH_3$$

Unlike the starting material, the product of this reduction contains an asymmetric carbon atom and it was found to be optically active. Since in the case of ketones a Cannizzaro reaction cannot take place, this mechanism for alcohol formation is out of the question. Consideration of the reduction of ketones thus clearly shows that an actual biohydrogenation is involved.

It is worthy of notice that acetaldehyde, a characteristic product of simultaneous oxidation in the fermentation mixture, was detected in these experiments. Its appearance is undoubtedly connected with the process of reduction. Acetaldehyde, which is formed in the absence of air and cannot therefore be a result of secondary oxidation, occurs in approximately constant ratio to the equivalent amount of the methylheptenol. An obvious relationship thus exists between the hydrogenation and the formation of the acetaldehyde. Presumably a disturbance of the normally correlated processes takes place, in the sense of the theory of fermentation. Whereas acetaldehyde normally is reduced to ethyl alcohol, in this case the intermediary acetaldehyde and the added ketone compete for the hydrogen. It thus follows that at least in the initial stage the amount of acetaldehyde which is not reduced must equal the amount that is displaced as a result of interference by the ketone.

The question whether similar conditions also play a part during the reduction of aldehydes must be left in abeyance. Such a mechanism cannot be postulated offhand, since an amount of acetaldehyde equivalent

(43) C. Neuberg and A. Lewite, *Biochem. Z.*, **91**, 257 (1918).

to the alcohol formed is in most cases difficult to determine. It would to conceivable, indeed, that the acetaldehyde is removed by dismutation or other reactions, as for example the formation of acyloins or their products of transformation (see pp. 77 and 86).

2. *Conversion of Various Aliphatic, Aromatic and Cylic Ketones*

Ketones, in contrast to aldehydes, occur frequently in plants. A phytochemical reduction of the keto group has been shown in aliphatic, aromatic[44] and cyclic ketones although it takes longer and is less complete than in the case of aldehydes. (For the theory of this transformation see Neuberg and Gorr.[45])

Neuberg and collaborators chose for these reductions the action of living and active yeast. Larger amounts were required than were necessary for aldehydes, but methyl ethyl, methyl propyl, methyl hexyl and methyl nonyl ketones and acetophenone could be converted in this way to their respective secondary alcohols. The biological character of the reaction again can be inferred from the fact that the resulting alcohols always showed optical activity. In spite of prolonged digestion with large amounts of sugar and yeast, a percentage of the starting material remained unaltered. Due to the similarity of the boiling points of ketones and alcohols, separation of the secondary alcohol from the corresponding ketone can best be achieved by chemical means. Either the unchanged ketone is removed as its bisulfite addition compound or, if this compound does not form completely, as in the case of many higher ketones, the removal is accomplished by treatment with hydrazines, particularly nitrophenylhydrazines.[46]

As already mentioned, the secondary alcohols that are obtained are optically active. It should be stressed that the reduction of ketones to carbinols by means of fermenting yeast is completely different from the method of resolution of racemic alcohols by treatment with living microorganisms (Pasteur). In the latter case one of the enantiomorphs is removed by oxidation during metabolism; in the former it is produced by true asymmetric hydrogenation, without the intermediate formation of the inactive form. (*Cf.* Mayer[47] and Levene and Walti.[38])

Phytochemical reduction has also been carried out successfully with a representative of the hydroaromatic series, namely, *d,l*-2-methylcyclohexanone. A strongly dextrorotatory 2-methylcyclohexanol was

(44) C. Neuberg and F. F. Nord, *Ber.*, **52,** 2237 (1919).
(45) C. Neuberg and G. Gorr, *Biochem. Z.*, **166,** 444 (1925).
(46) C. Neuberg and E. Reinfurth, *Biochem. Z.*, **89,** 398 (1918).
(47) P. Mayer, *Biochem. Z.*, **174,** 420 (1926).

obtained in 38% yield.[48] Cyclopentanone is converted to cyclopentanol by fermenting bottom yeast.[49]

3. *Phytochemical Reduction of Hydroxy Ketones*

Due to the close relationship between certain α-hydroxy ketones, the acyloins and α-diketones, their behaviors during phytochemical reduction have been treated together (see pp. 86–89).

Using as an example the lowest member of the series of hydroxy ketones, acetol, $CH_3\cdot CO\cdot CH_2OH$, it can be shown that in this series, too, phytochemical reduction to the corresponding propylene glycol can be achieved readily.[50] In view of its relationship to methylglyoxal, lactic acid, pyruvic acid and sugars, the three-carbon compound acetol is worthy of special consideration. Since the 1,2-propylene glycol that was obtained by its reduction was always levorotatory, $[\alpha]_D$ $-14.6°$, the asymmetric course of the phytochemical reduction is again established. No difficulties were encountered in the hydrogenation of acetol, which was prepared according to the directions of Nef.[51] With fermenting top yeast the reduction was completed in three to five days but the concentration of acetol never exceeded 1 to 1.6%. This method is also applicable to aliphatic keto alcohols of higher molecular weight. Veibel[52] reduced 4-hydroxy-5-octanone, $CH_3\cdot CH_2\cdot CH_2\cdot CO\cdot CHOH\cdot CH_2\cdot CH_2\cdot CH_3$, to 4,5-octanediol. This glycol, $CH_3\cdot CH_2\cdot CH_2\cdot CHOH\cdot CHOH\cdot CH_2\cdot CH_2\cdot CH_3$, consisted of the meso form and the strongly dextrorotatory form. Conditions thus correspond to those of the bacterial formation of 2,3-butylene glycol; there, too, a meso form and an optically active form result.[53] It is thus shown that phytochemical reduction extends to hydroxyketones the carbonyl group of which is not in the α position.

In claret wine turned bitter, Voisenet[53a] observed a divinylglycol, $CH_2{=}CH\cdot CHOH\cdot CHOH\cdot CH{=}CH_2$. It is not known which isomer is involved and whether the compound is optically active. The glycol is considered to be derived from acrolein. It could originate either by a reduction comparable to the formation of pinacol from acetone or by bioreduction of an acyloin-like condensation product of acrolein.

(48) S. Akamatsu, *Biochem. Z.*, **142**, 188 (1923).
(49) C. Neuberg and E. Peiser, unpublished.
(50) E. Färber, F. F. Nord and C. Neuberg, *Biochem. Z.*, **112**, 313 (1920).
(51) J. U. Nef, *Ann.*, **335**, 260 (1904).
(52) S. Veibel, *Biochem. Z.*, **239**, 456 (1931).
(53) G. S. Walpole, *Proc. Royal Soc. London*, **B83**, 272 (1911); J. Böeseken and R. Cohen, *Rec. trav. chim.*, **47**, 839 (1928); M. Lemoigne and P. Monguillon, *Compt. rend.*, **190**, 1457 (1930); **191**, 80 (1930).
(53a) E. Voisenet, *Compt. Rend.*, **188**, 1271 (1929).

Levene and Walti[54] also reduced phytochemically 1-hydroxy-3-butanone to the levorotatory 1,3-butanediol and 1-hydroxy-2-heptanone to the dextrorotatory 1,2-heptanediol.[38,5] It seems[55] that the optically active glycols that are obtained by bioreduction of hydroxy ketones with fermenting yeast are configurationally related. But the 1,3-butanediol that is obtained by the reduction of the 1-hydroxy-3-butanone[38] has the opposite configuration from the product of bioreduction of the isomeric *d,l*-acetaldol[36] (see p. 81).

It remains undecided whether the formation of small amounts of glycerol reported by Oppenheimer[55a] in the case of zymase extract is due to a phytochemical reduction of trioses. If hexoses are fermented in the presence of trioses with ordinary fresh yeasts which do not attack dihydroxyacetone and glyceraldehyde, the added trioses are recovered practically unaltered after the disappearance of the hexoses.

4. *Phytochemical Reduction of Keto Acids and Keto Aldehydes*

Phytochemical reduction of oxalacetic (α-ketosuccinic) acid to optically active malic acid occupies today an important place in the mechanism of several metabolic processes; it was first demonstrated years ago by Neuberg and Gorr,[56] Mayer[57] and Fujise.[58] The analogous reduction of acetoacetic acid to β-hydroxybutyric acid has been reported by Friedmann;[59] if this reduction is carried out with yeast, the dextrorotatory form of β-hydroxybutyric acid results, while it is well known that the levorotatory form is obtained by means of animal cells and also, according to Heitzmann,[60] with the *Bacillus M* of Lemoigne. The reduction of acetoacetaldehyde by fermenting yeast[61] yields levorotatory 1,3-butylene glycol, analogous to the phytochemical reduction of methylglyoxal, which results in levorotatory propylene glycol accompanied by D(*levo*)-lactic acid.[62]

5. *Phytochemical Reduction of Halogenated Ketones*

Examples of the reduction of substances of this type have been discussed on page 81.

(54) P. A. Levene and A. Walti, *J. Biol. Chem.*, **98**, 735 (1932).

(55) P. A. Levene and H. L. Haller, *J. Biol. Chem.*, **76**, 418 (1928).

(55*a*) M. Oppenheimer, *Z. physiol. Chem.*, **89**, 68 (1915).

(56) C. Neuberg and G. Gorr, *Biochem. Z.*, **164**, 495 (1925); *Ergeb. Physiol.*, **24**, 191 (1925).

(57) P. Mayer, *Biochem. Z.*, **156**, 300 (1925). See also J. K. Parnas and W. Szankowski, *Enzymologia*, **3**, 220 (1937).

(58) S. Fujise, *Biochem. Z.*, **236**, 231 (1931).

(59) E. Friedmann, *Biochem. Z.*, **243**, 125 (1931).

(60) P. Heitzmann, *Compt. rend.*, **214**, 509 (1942).

(61) S. Grzycki, *Biochem. Z.*, **265**, 195 (1933).

(62) C. Neuberg and Maria Kobel, *Biochem. Z.*, **182**, 472 (1927).

IV. The Phytochemical Reduction of Diketones and Quinones

After phytochemical reduction was noted in the case of aldehydes and ketones, interest arose in the behavior of fermenting cells toward compounds containing several carbonyl groups per molecule, such as diketones and quinones. This class deserves special consideration because the simplest representative, diacetyl, as well as its products of reduction, acetylmethylcarbinol (3-hydroxybutanone) and 2,3-butylene glycol, are connected with the metabolism of numerous cells; quinones also are biologically important.

The first experiments made by Neuberg and Nord[63] with the simplest diketone, diacetyl, showed at once that this substance can be hydrogenated phytochemically with comparitive ease. Acetylmethylcarbinol appears as an intermediate (see below), and the end product of reduction is asymmetric and levorotatory. Reduction was effected by the action of fermenting yeast on diacetyl. The 2,3-butanediol that is formed can be isolated by alcohol-ether extraction of the fermentation mixture after concentration in the Faust-Heim apparatus or by steam distillation in an atmosphere of carbon dioxide under ordinary pressure; it is then carefully concentrated with the birectifier and obtained in the pure state by final fractionation.

According to Nagelschmidt[64] acetylmethylcarbinol is an intermediate in the phytochemical reduction of diacetyl, representing a partial hydrogenation of the diketone. It had already been demonstrated by Neuberg and Gorr[56] that acetoin, which is carboligatically produced by the sugar-free fermentation of oxalacetic acid, can be converted to 2,3-butylene glycol by means of yeast. Neuberg and Kobel[65] successfully accomplished the phytochemical reduction of synthetically prepared *d,l*-acetylmethylcarbinol to levorotatory 2,3-butylene glycol in 66% yield.

Acetylmethylcarbinol has also been recognized as a product of the metabolism of animal cells[66] and its origin from sugar by way of pyruvic acid and acetaldehyde has been demonstrated. It therefore appears probable that 2,3-butylene glycol, which together with acetylmethylcarbinol

(63) C. Neuberg and F. F. Nord, *Ber.*, **52,** 2248 (1919).

(64) G. Nagelschmidt, *Biochem. Z.*, **186,** 317 (1927).

(65) C. Neuberg and M. Kobel, *Biochem. Z.*, **160,** 250 (1925).

(66) G. Gorr, *Biochem. Z.*, **254,** 12 (1932); G. Gorr and J. Wagner, *ibid.*, **254,** 5, 8 (1932); A. Stepanow and A. Kusin, *Ber.*, **67,** 721 (1934); H. Schmalfuss, Wilhelmina Hinsch and Helene Schmalfuss, *Z. physiol. Chem.*, **227,** 247 (1934); D. E. Green, W. W. Westerfeld, B. Vennesland and W. E. Knox, *J. Biol. Chem.*, **140,** 683 (1941), **145,** 69 (1942); E. Stotz, W. W. Westerfeld and R. L. Berg, *ibid.*, **152,** 41 (1944); R. L. Berg and W. W. Westerfeld, *ibid.*, **152,** 113 (1944).

is also found in higher plants and in the animal organism,[67] is formed in a manner analogous to phytochemical reduction.

It is possible, although not proven, that the remarkable fermentation of 5-ketofructose (5-D-fructonose),

$$CH_2OH—CO—\overset{\overset{H}{|}}{\underset{\underset{OH}{|}}{C}}—\overset{\overset{OH}{|}}{\underset{\underset{H}{|}}{C}}—CO—CH_2OH,$$

involves the previous phytochemical reduction of one carbonyl group, which results in the formation of D-fructose.[68]

The occurrence of acetylmethylcarbinol as an intermediate of the biochemical hydrogenation of diacetyl is analogous to the conversion of the simplest aromatic diketone, benzil.[69] Under corresponding conditions the reaction stops at the half-hydrogenated stage. The main product derived from benzil, $C_6H_5·CO·CO·C_6H_5$, is benzoin, $C_6H_5·CHOH·CO·C_6H_5$, while the formation of the glycol (hydrobenzoin) could not be definitely established. The fact that benzoin itself was hardly attacked by fermenting yeast shows at least that there was no pronounced tendency to transform benzoin to hydrobenzoin under the existing conditions. Incidentally, small amounts of an optically active substance do adhere to the benzoin that is formed by phytochemical reduction of benzil; presumably this substance is an admixture of the known[70] levorotatory form of benzoin. Theoretically it seems significant that the reducing agents attack the difficultly soluble benzil, which to a large extent remains suspended in the fermentation mixture in the form of crystals, and that the product of hydrogenation is likewise of low solubility. The formation of acetaldehyde in appreciable amounts could again be observed to accompany these products of reduction. Hydrogenation of diketones thus apparently takes the same course as that suggested for the transformation of simple ketones (see p. 82).

A product of carboligatic synthesis, that is, the biological acyloin condensation, phenylacetylcarbinol, $CH_3·CO·CHOH·C_6H_5$ (3-phenyl-3-hydroxypropanone), undergoes phytochemical reduction[71] to α-methyl-β-phenyl-ethylene glycol, $CH_3·CHOH·CHOH·C_6H_5$. According to Neuberg and Komarewsky,[72] the *d,l* form of methylbenzoylcarbinol,

(67) M. Lemoigne and P. Monguillon, *Compt. rend.*, **190**, 1457 (1930); **191**, 80 (1930); Y. Tomiyasu, *Enzymologia*, **3**, 263 (1937).

(68) F. Micheel and K. Horn, *Ann.*, **515**, 6 (1935).

(69) C. Neuberg and F. F. Nord, *Ber.*, **52**, 2251 (1919).

(70) A. McKenzie and H. Wren, *J. Chem. Soc.*, **93**, 309 (1908); H. Wren, *ibid.*, **95**, 1583 (1909).

(71) C. Neuberg and H. Ohle, *Biochem. Z.*, **128**, 610 (1922).

(72) C. Neuberg and W. Komarewsky, *Biochem. Z.*, **182**, 285 (1927).

$CH_3 \cdot CHOH \cdot CO \cdot C_6H_5$, is converted by phytochemical reduction readily and in good yield to the levorotatory α-methyl-β-phenyl-ethylene glycol. Under appropriate conditions[73] this reaction can be made to yield the levorotatory α-methyl-β-phenyl-ethylene glycol, leaving the dextrorotatory hydroxy ketone unchanged. The glycol that is produced is identical with the one that is obtained from phenylacetylcarbinol through an entirely phytochemical reduction. By careful oxidation with nitric acid the glycol may be converted to the levorotatory hydroxy ketone.

This reaction shows that during phytochemical reduction of a racemic compound one of the optical isomers may be attacked preferentially to such an extent that the optical enantiomorph remains unaltered. In this process the enzyme shows a great specificity for one of the two possible enantiomorphous hydrogen acceptors. This behavior is in accord with the theory of biochemical transfer of hydrogen atoms put forward by Wieland.[74] Alternatively, it may be considered in the light of the explanations given by Verley[75] and by Meerwein and Schmidt,[76] who studied the mechanism of processes taking place between hydrogen acceptor and donor through the formation of special addition products. Other mechanisms have also been suggested.[77] (See also the phenomena of the crossed Cannizzaro reaction and dismutation on pp. 101 and 102.)

When a racemic substance is hydrogenated or when the reduction leads to the production of centers of asymmetry, the phytochemical reduction will take at first a completely or partially asymmetric course. Examples of such asymmetric reactions are the conversions of pure racemic valeraldehyde, acetaldol, furoin and furil, diacetyl and acetylmethylcarbinol to optically active alcohols. Occasionally meso forms also arise, as for example in the case of glycols (p. 84). The reasons for the stereochemical specificity of these reactions have not been clarified.[43] This type of phenomenon has frequently been observed in the related intramolecular dismutation of keto aldehydes, especially if enzyme materials of differing origins are used.

Animal tissues are capable of carboligatically joining propionaldehyde and acetaldehyde to synthesize the homolog of acetylmethylcarbinol,[66] propionylmethylcarbinol, $CH_3 \cdot CHOH \cdot CO \cdot CH_2 \cdot CH_3$. Yeast attacks the corresponding diketone, acetylpropionyl, $CH_3 \cdot CO \cdot CO \cdot CH_2 \cdot CH_3$, and yields two stereoisomeric glycols with the formula $CH_3 \cdot CHOH \cdot CHOH \cdot$-$CH_2 \cdot CH_3$. At first the dextrorotatory form is present in excess. Just

(73) F. von Falkenhausen, *Biochem. Z.*, **219**, 241 (1930).
(74) H. Wieland, *Ber.*, **46**, 3341 (1913).
(75) A. Verley, *Bull. soc. chim.*, [4] **41**, 788 (1927).
(76) H. Meerwein and R. Schmidt, *Ann.*, **444**, 229 (1925).
(77) H. Fredenhagen and K. F. Bonhöffer, *Z. physik. Chem.*, [A] **181**, 379 (1938).

as in the case of the biohydrogenation of diacetyl (see p. 86), a ketopentanol is formed intermediarily. The isomeric acetylacetone, $CH_3 \cdot CO \cdot CH_2 \cdot CO \cdot CH_3$, is hydrogenated only slowly and incompletely by fermenting yeast.[78]

The phytochemical reducibility of quinones was first demonstrated[79] in the case of *p*-xyloquinone. This compound is worthy of interest since it is very easily formed from diacetyl by purely chemical means through a type of aldol condensation followed by ring closure. It is reduced to *p*-xylohydroquinone by fermenting yeast.[79] Benzoquinone, thymoquinone and α-naphthoquinone similarly yield the corresponding hydroquinones. Tetrabromo-*o*-quinone and anthraquinone proved resistant to attack, while phenanthraquinone could be reduced phytochemically to phenanthrahydroquinone in poor yield (9%).[80] Phytochemical reduction can also be accomplished in the dicyclic terpene series. According to unpublished experiments by Neuberg and Peiser, 2,3-dihydroxycamphane (camphorquinone), $C_8H_{14}\left\langle\begin{array}{l}CO\\ |\\ CO\end{array}\right.$, is reduced in 63% yield to 3-hydroxycamphor, $C_8H_{14}\left\langle\begin{array}{l}CHOH\\ |\\ CO\end{array}\right.$; *dextro*-camphorquinone gives dextrorotatory hydroxycamphor, *d,l*-camphorquinone a levorotatory hydroxy compound. Thus an *o*-quinone could be phytochemically reduced to a satisfactory extent. As in the case of benzil (see p. 87), only one carbonyl group is reduced under these conditions.

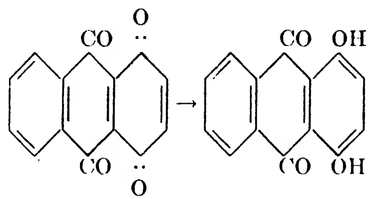
V. The Phytochemical Reduction of Polycarbonyl Compounds

Triketopentane, $CH_3 \cdot CO \cdot CO \cdot CO \cdot CH_3$, which itself is one of the strongest purely chemical reducing agents, is reduced phytochemically to symmetrical 1,3-dimethylglycerol, $CH_3 \cdot CHOH \cdot CHOH \cdot CHOH \cdot CH_3$. While this product is optically inactive,[81] optically active substances still containing carbonyl groups are byproducts of the reaction, formed by partial reduction of the triketone. This triketone is deep yellow in color but during the phytochemical reduction the liquid soon becomes color-

(78) S. Veibel and Erna Bach, *Kgl. Danske Videnskab. Selskab, Math.-fys. Medd.*, **13**, No. 18 (1936); *Chem. Abstracts*, **30**, 5717 (1936).
(79) C. Neuberg and E. Simon, *Biochem. Z.*, **171**, 256 (1926).
(80) H. Lüers and J. Mengele, *Biochem. Z.*, **179**, 238 (1926).
(81) C. Neuberg and W. M. Cahill, *Enzymologia*, **1**, 142 (1936).

less. It is interesting to note that in spite of its strong chemical affinities the triketone exhibits very little poisonous action on the yeasts.

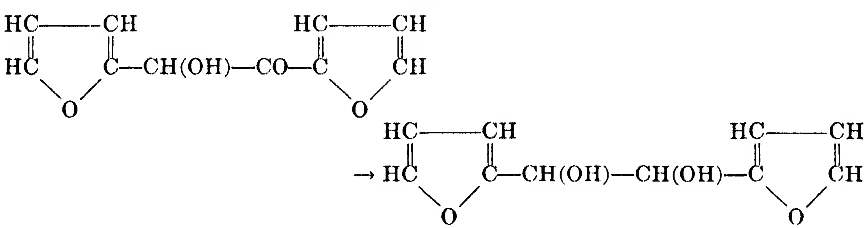
A tetraketone, 1,4,9,10-anthradiquinone, is reduced by fermenting yeast to quinizarin:[82]



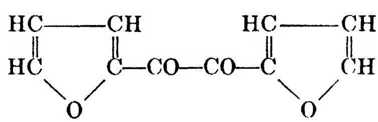
The reduction thus extends to the two carbonyl groups in the outer part of the anthraquinone nucleus, but not to the meso carbonyl groups. The picture of the phytochemical reduction is obscured by the fact that anthradiquinone is rather easily decomposed and undergoes autoreduction to quinizarin by partial oxidation and ring cleavage of a second molecule to phthalic acid.

VI. The Phytochemical Reduction of Heterocyclic Compounds

Neuberg, Lustig and Cagan[83] were able to reduce furoin to hydrofuroin by the action of fermenting yeast:



Furil,



forms the same glycol by way of the intermediate furoin. The products show optical activity. This biochemical reaction is worthy of note because the purely chemical reductions of furoin or furil have not been successful,[84] desoxyfuroin being produced instead of the glycol.

(82) A. Vercellone, *Biochem. Z.*, **279**, 137 (1935).

(83) C. Neuberg, H. Lustig and R. N. Cagan, *Arch. Biochem.*, **1**, 391 (1943). Regarding the course of biochemical transformations of furfural (8,9,85) see also newer data by C. Neuberg, H. Lustig and R. Dresel, *Arch. Biochem.*, **19**, 163 (1948).

(84) W. C. Albert and A. Lowy, *Trans. Electrochem. Soc.*, **75**, 367 (1939).

The formation of furoin from furil represents *the simplest and quickest demonstration of phytochemical reduction for lecture purposes.* The presence of the acyloin can be demonstrated, even in extreme dilution, by its characteristic reaction with aqueous or alcoholic sodium hydroxide (deep blue-green color with deep violet-red dichroic irridescence). This reaction can be carried out in unfiltered fermentation mixtures; it gives positive results after thirty seconds. The addition of a few crystals of commercially available furil dissolved in one cc. alcohol to a fermenting sugar solution is sufficient for demonstration.[83]

The phytochemical reduction of acetylfurylcarbinol takes place so rapidly that it cannot be isolated during carboligatic formation of this acyloin, the corresponding methylfurylethylene glycol being obtained directly.[85]

2-Thiophene-carbinol is synthesized from 2-thiophene aldehyde by fermenting yeast.[86] The behavior of 2,2′ thenoin and 2,2′ thenil on bioreduction is exactly analogous to that of furoin and furil.[86a]

VII. The Phytochemical Reduction of Unsaturated Ethylenic Linkages

Under ordinary conditions phytochemical reduction of unsaturated compounds yields unsaturated subtances (see p. 79). In experiments made with cinnamyl aldehyde,[28] $C_6H_5{\cdot}CH{=}CH{\cdot}CHO$, the reduction yielded cinnamyl alcohol, $C_6H_5{\cdot}CH{=}CH{\cdot}CH_2OH$, but there was indication, though not positive proof, that some hydrocinnamyl alcohol, $C_6H_5{\cdot}CH_2{\cdot}CH_2{\cdot}CH_2OH$, was also produced. According to later investigations by Fischer and Wiedemann,[87] double bonds of certain compounds of this type may be reduced by yeast, especially when conditions for vigorous fermentation are provided; in particular cases they obtained the following results. Cinnamaldehyde was extensively reduced to hydrocinnamyl alcohol and a small amount of cinnamyl alcohol. Crotonaldehyde, $CH_3{\cdot}CH{=}CH{\cdot}CHO$, yielded *n*-butyl alcohol and some crotyl alcohol, $CH_3{\cdot}CH{=}CH{\cdot}CH_2OH$. Sorbic aldehyde, $CH_3{\cdot}(CH{=}CH)_2{\cdot}CHO$, yielded the hexenol $CH_3{\cdot}CH{=}CH{\cdot}CH_2{\cdot}CH_2{\cdot}CH_2OH$ and sorbyl alcohol, $CH_3{\cdot}(CH{=}CH)_2{\cdot}CH_2OH$. Sorbylidenepyruvic acid, $CH_3{\cdot}(CH{=}CH){\cdot}_3{\cdot}CO{\cdot}COOH$, yielded octatrienol, $CH_3{\cdot}(CH{=}CH)_3{\cdot}CH_2OH$, and octatrienic acid, $CH_3{\cdot}(CH{=}CH)_3{\cdot}COOH$; thus, ordinary dismutation took place after previous carboxylatic cleavage; with strongly fermenting yeast, however, there were produced octadienol, $CH_3{\cdot}(CH{=}CH)_2{\cdot}CH_2{\cdot}CH_2{\cdot}CH_2OH$, and the triply unsaturated octatrienol. In an analogous way benzylidenepyruvic acid, $C_6H_5{\cdot}CH{=}CH{\cdot}CO{\cdot}COOH$, underwent decar-

(85) P. Liang, *Z. physiol. Chem.*, **244**, 238 (1936).
(86) F. W. Dunn and K. Dittmer, *J. Am. Chem. Soc.*, **68**, 2561 (1946).
(86a) I. Deschamps *et al.*, *J. Org. Chem.*, **14**, 184 (1949).
(87) F. G. Fischer and O. Wiedemann, *Ann.*, **513**, 265 (1934).

boxylation and hydrogenation to yield hydrocinnamyl alcohol. Dihydro alcohols were formed in varying yields from the unsaturated crotyl alcohol, sorbyl alcohol and cinnamyl alcohol. The double bond of 2-methyl-γ-hydroxy-2-heptene, $CH_3{\cdot}CH(OH){\cdot}CH_2{\cdot}CH_2{\cdot}CH{=}C(CH_3)_2$, proved to be resistant, in accord with previous[43] observations. The phytochemical reduction of α,β-unsaturated ketones leads predominantly to the saturated ketones; afterwards there may occur a slow reduction of the carbonyl group to yield the respective secondary alcohols. Thus, benzylideneacetone, $C_6H_5{\cdot}CH{=}CH{\cdot}CO{\cdot}CH_3$, is transformed within eight days to benzylacetone, and 1-methylcyclohexen-3-one gives rise to dextrorotatory 1-methyl-3-cyclohexanol (*synonym:* 3-methylcyclohexanol). On the other hand, mesityl oxide, pulegone and carvone are hardly attacked at all. From allylacetone (1-hexen-5-one), $CH_2{=}CH{\cdot}CH_2{\cdot}CH_2{\cdot}CO{\cdot}CH_3$, there was formed in moderate yield 1-hexen-5-ol by reduction of the keto group; in this case the double bond remained intact. On the whole it has been found that ethylene linkages may be reduced phytochemically especially when they are conjugated with a carbonyl group. Thus dienes are only reduced in the 1,2 positions; in conjugated trienes hydrogen is taken up solely in the 1,2 position. If an asymmetric carbon atom is formed, the products of reduction generally show optical activity (see pp. 80, 81, 88). In this way tiglic aldehyde yields levorotatory amyl alcohol, and citral or geraniol is reduced to dextrorotatory citronellol. This asymmetric course was observed by Fischer[88] for living yeast only; when cell-free maceration juice is applied, racemic mixtures are formed. Analogous results have been reported concerning the different stereochemical behavior of living microbes and enzymes separated from them.[89]

Ideas regarding the mechanism of phytochemical reduction of olefins have been advanced by Fischer and Eysenbach.[90] They also point out that just as in the case of the simple disproportionations in the course of sugar degradation (for example, the second and third forms of fermentation), saturation of the olefinic linkage is at an optimum in the weakly alkaline range, at about pH 8.5.

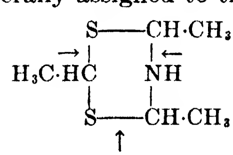
The first application of the manifold experiences gained in the field of bioreductions to substances of the cholic acid and sterol series is due to Kim.[91] He showed that dehydrodesoxycholic acid is transformed by bottom yeast to 3-hydroxy-12-ketocholanic acid. Mamoli and Vercel-

(88) F. G. Fischer, *Z. angew. Chem.*, **49**, 560 (1936).
(89) C. Neuberg and E. Simon, *Biochem. Z.*, **186**, 333 (1927).
(90) F. G. Fischer and H. Eysenbach, *Ann.*, **529**, 89 (1937); **530**, 99 (1937).
(91) C. H. Kim, *Enzymologia*, **4** (Neuberg Festband), 119 (1937); **6**, 105 (1939).

lone[92] demonstrated that certain unsaturated cyclic ketones of the sterol series can also be reduced phytochemically. α-Estradiol is formed in 60% yield from estrone (used as its acetate). Δ^5-Dehydroandrosterone gives Δ^5-androstenediol; Δ^5-androstenedione yields, beside Δ^4-testosterone, mainly isoandrostanediol. Δ^4-Androstenedione gives Δ^4-testosterone. The conclusion arrived at by these authors—that in the steroid series a double bond in conjugation with the carbonyl group prevents phytochemical reduction of the latter—must be modified. Beside the structure of the side chain the position of the double bond conjugated with a carbonyl group has a decisive influence. According to Butenandt and co-workers[93] Δ^1-androstene-17-ol-3-one and Δ^1-androstene-3,17-dione are both converted to isoandrostane-3,17-diol. Furthermore, Δ^1-androstenedione-3,17 yields Δ^1-androstene-17-ol-3-one.

VIII. The Phytochemical Reduction of Thioaldehydes and Disulfides

After a series of facts had made it appear probable that alcohols are formed by way of the aldehydes during various processes of metabolism, experiments were set up to ascertain whether thioalcohols (mercaptans) are formed in an analogous manner. Neuberg and Nord[94] found that phytochemical reduction converts thioacetaldehyde very readily into ethyl mercaptan. The difficulty lay in employing a suitable form of the thioacetaldehyde; while the monomolecular thioaldehyde is known to be unstable, the stable trimolecular form, believed to have a ring structure, is practically insoluble in the solvents employed. By using the sulfated aldehyde ammonia, namely thialdine, these difficulties may be circumvented. The formula generally assigned to this compound,



shows that through cleavage as indicated by the three arrows it can supply two molecules of thioacetaldehyde and one molecule of aldehyde ammonia. Since thialdine readily dissolves in alcohol it can be added to the fermentation mixture without any trouble. If a thialdine solution is

(92) L. Mamoli and A. Vercellone, *Ber.*, **70**, 470, 2079 (1937), **71**, 2696 (1938), *Z. physiol. Chem.*, **245**, 93 (1937), **248**, 277 (1937). See also A. Wettstein, *Helv. Chim. Acta*, **22**, 250 (1939).

(93) A. Butenandt and H. Dannenberg, *Ber.*, **71**, 1681 (1938); A. Butenandt, H. Dannenberg and L. A. Surányi, *ibid.*, **73**, 818 (1940).

(94) C. Neuberg and F. F. Nord, *Ber.*, **47**, 2264 (1914), *Biochem. Z.*, **67**, 46 (1914).

added to a fermenting sucrose solution fermentation is not significantly inhibited. After a lapse of about five minutes the fermentation gases begin to emit a disagreeable odor, which is easily recognized as that of mercaptan. While the presence of mercaptan thus can be demonstrated easily in a qualitative way, its quantitative determination is difficult. Ethyl mercaptan boils at 36°. It is formed gradually during the course of the fermentation and is always considerably diluted with the carbon dioxide, that is developed at the same time.

The following method was used for the quantitative determination of ethyl mercaptan. The gases given off during fermentation are conducted through a system of wash bottles filled with 3% mercuric cyanide solution. After a few minutes a gradually increasing yellow precipitate is formed in the first bottle. Since pure mercuric mercaptides are white, the yellowish tint must be caused by impurities. In the course of fermentation, mercuric sulfide is formed in the wash bottle along with the mercaptide. This sulfide arises from the liberation of hydrogen sulfide, caused by the simple action on the thialdine of the carbon dioxide formed during fermentation. Furthermore, since the mercuric ethyl mercaptide seems to decompose under the influence of light to give mercury or mercuric sulfide, the procedure followed was to remove the bottle adjoining the fermentation vessel as soon as a thick precipitate of insoluble mercury compounds had formed, to stopper it well and to place it in the dark. The fermentations were conducted in a room at ordinary temperature but the fermentation vessel was immersed in a water bath, which during the day was refilled with warm water at 40° every two hours. After three days all the sugar had fermented. A special distillation was unnecessary since it could be presumed that the ethyl mercaptan had volatilized with the fermentation carbon dioxide. Finally, more carbon dioxide was passed into the system from a carbon dioxide bomb. Occasionally this gas was also passed through the apparatus during the course of fermentation, particularly if the normal rate of flow was low because of the pressure produced by abnormally large amounts of the absorbent. All precipitates were collected and washed with cold water; after treatment with 5% hydrochloric acid, or better with 10% phosphoric acid, the mercaptans were distilled. The distilling vessel was connected through a condenser with an absorption column containing 3% lead acetate solution. On careful heating, a gas was liberated which produced a yellow precipitate with the absorbent. After the material had stood in the refrigerator for some time, the precipitate was filtered off by suction and washed with water and alcohol. Analysis led to the conclusion that the substance was the pure lead salt of ethyl mercaptan. The yield obtained from 20 g. thialdine was 1.3 g. of lead mercaptide.

Experiments made with yeast that had been killed by boiling showed that even in the presence of sugar such yeast is unable to reduce thioacetaldehyde to ethyl mercaptan.

The formation of mercaptan is doubly important because of its relation to phytochemical reduction processes. The reaction is analogous to the formation of ordinary alcohols from aldehydes and it also indicates how the plant is capable of synthesizing intensely odorous substances from aldehydes and hydrogen sulfide in a simple manner. The isolation

of ethyl mercaptan by the method described above was the first case in which this substance was unequivocally prepared by a fermentation process.

In view of the specific nature of the whole process it appeared interesting to investigate whether the reduction of thioacetaldehyde to mercaptan could also be accomplished with cell-free enzyme solutions. Neuberg and Nord[94] found that yeast maceration juice effects this hydrogenation (see p. 79).

This method of phytochemical reduction may also be extended to homologs of thioacetaldehyde. In this way Nord[95] converted the ammonia derivatives of *n*-thiobutyraldehyde and thioisovaleraldehyde to *n*-butyl and isoamyl mercaptans, respectively.

In such experiments, a preliminary preparation of the often difficulty obtainable pure thioaldehydes is not actually necessary. It suffices to bring the fermenting yeast in contact with the ordinary (non-thio) aldehydes and with ammonia and hydrogen sulfide (ammonium hydrogen sulfide). Such conditions frequently apply for animals and plants, for example, in bacteriological processes and probably also in the metabolism of higher organisms.

It appeared probable that the reduction of disulfides to mercaptans by means of yeast would be particularly easy, since the two substances can be interconverted quite smoothly by ordinary chemical means. This prediction did not prove to be quite correct. True, Neuberg and Schwenk[96] could reduce a disulfide by means of yeast, but they could not do so with the expected ease. Ethyl disulfide was chosen for the experiment because its boiling point (151°) differs very much from that of ethyl mercaptan (36°). Yeast which has been killed by boiling does not convert the disulfide into the mercaptan, but fermenting yeast does; in view of the physiological importance of the disulfide group in cystine and glutathione, this observation is worthy of note.

IX. The Phytochemical Conversion of Thiosulfate into Hydrogen Sulfide and Sulfite[97] and the Bioreduction of Other Inorganic Compounds

It has long been known that sulfur can be reduced to hydrogen sulfide by means of yeast (see p. 76). Strangely enough, only scanty information existed regarding the behavior of yeasts toward thiosulfates even though the unusually bound sulfur atom of these compounds may

(95) F. F. Nord, *Ber.*, **52**, 1207 (1919).
(96) C. Neuberg and E. Schwenk, *Biochem. Z.*, **71**, 118 (1915).
(97) C. Neuberg and E. Welde, *Biochem. Z.*, **67**, 111 (1914).

be assumed to have aroused interest. Beijerinck[98] noticed an action of bacteria of the *Aerobacter* group on thiosulfate (besides tetra- and penta-thionate). Bokorny[99] observed a very small quantity of hydrogen sulfide, only just detectable by its odor, when yeast was cultivated in a medium containing sodium thiosulfate. He was inclined, however, to ascribe this hydrogen sulfide to the action of contaminating bacteria. A more detailed investigation of this subject has been made by Kossowicz and Loew.[100] If various types of *Saccharomyces* are cultivated in suitable sugar-containing nutrient solutions a slight action on added thiosulfate can be detected after three to four weeks. As growth of the yeast increases, a minute development of hydrogen sulfide starts, which can be determined only after some weeks have elapsed. The titration showed but a very small decrease in the amount of thiosulfate, even in cases in which a production of hydrogen sulfide could be detected. Apparently these early experiments were conducted partly in a qualitative way only, with growing but not fermenting yeast. In view of the fact that the reducing power of yeast is especially enhanced in fermenting sugar solution, Neuberg and Welde[97] investigated the action of active yeasts on thiosulfates. If the gases given off during such fermentation are led through a system of wash bottles with suitable absorption liquids which take up hydrogen sulfide but not carbon dioxide (copper sulfate, silver nitrate or cadmium acetate solutions) the amount of hydrogen sulfide liberated can be determined easily. In two to four days the authors were able to obtain 16% of the theoretically possible amount of this substance.

The calculation is based on the fact that the hydrogen sulfide had been formed from the added sodium thiosulfate by a true reduction according to the equation:

$$Na_2S_2O_3 + H_2 = H_2S + Na_2SO_3$$

and not by simple hydrolysis according to the formulation:

$$Na_2S_2O_3 + H_2O = Na_2SO_4 + H_2S$$

The hydrolytic reaction would not have been of interest, while the reductive formation would bring the process in line with other analogous phytochemical reductions. The fact that the presence of sodium sulfite in the fermentation mixture has been established, while sulfate is absent, proves that a true hydrogenation does take place. Previously, it was not considered that two possibilities exist for this reaction. It has been ascertained analytically that the precipitate produced by the fermenta-

(98) M. W. Beijerinck, *Zentr. Bakt. Parasitenk. Abt. II*, **6**, 205 (1900).
(99) Th. Bokorny, *Zentr. Bakt. Parasitenk. Abt. II*, **35**, 141 (1912).
(100) A. Kossowicz and W. Loew, *Z. Gärungsphysiol.*, **2**, 87 (1913).

tion gases in the absorption flasks really contains pure metal sulfides. Since yeast which has been killed by boiling does not show any significant action on thiosulfate even in the presence of sugar, all these processes must necessarily involve the participation of fermenting yeast.

According to Garreau,[101] fermenting yeast liberates a small amount of hydrogen sulfide from cysteine and a little more from mercaptopyruvic acid. According to von Euler, Högberg and Gernow,[102] sulfur combines with organic constituents of yeast if the yeast is stirred with milk of sulfur. After this treatment, hydrogen sulfide develops on standing by a partly enzymatic, partly nonenzymatic mechanism.

The general possibility of sugar fermentation in the presence of hydrogen sulfide, even in a hydrogen sulfide atmosphere, was recognized at an early date.[103]

An observation of Nielsen, Shull and Peterson[104] may be connected with the process of reduction. These authors demonstrated that a substance obtained by mild oxidation of biotin with H_2O_2, in a lower state of oxidation than the sulfone of Hofmann, Melville and du Vigneaud,[105] can be utilized by yeast.

Apart from sulfur and sodium thiosulfate, numerous inorganic salts undergo phytochemical reduction, that is, such salts as are formed by elements capable of existing in various valence states and known as activators of fermentation.[106] The early fundamental investigations of Söhngen[107] and more recent publications by Mann and Quastel[108] demonstrate that this process must be considered in the case of the manganese cycle in soils. Bacterial reduction of manganese dioxide to divalent manganese is promoted by D-glucose.

X. The Phytochemical Conversion of Nitrogen-Oxygen Compounds and Other Nitro Compounds

Organic nitrogen compounds are of great importance in the vegetable kingdom, where they occur in the form of proteins, bases and alkaloids.

(101) Y. Garreau, *Compt. rend. soc. biol.*, **137,** 176 (1943).

(102) H. von Euler, B. Högberg and I. Gernow, *Arkiv Kemi, Mineral. Geol.*, No. 3 (1944); *Chem. Abstracts*, **39,** 3805 (1945).

(103) C. Neuberg and G. Perlmann, *Biochem. Z.*, **165,** 238 (1925).

(104) E. Nielsen, G. M. Shull and W. H. Peterson, *J. Nutrition*, **24,** 523 (1942).

(105) K. Hofmann, D. B. Melville and V. du Vigneaud, *J. Biol. Chem.*, **141,** 207 (1941).

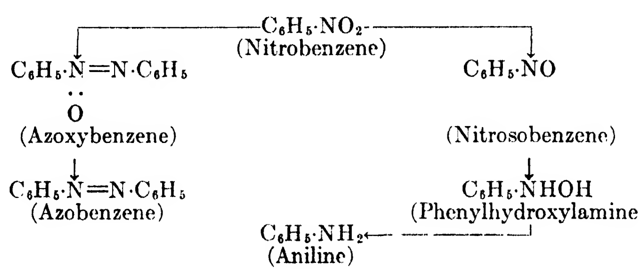
(106) C. Neuberg and M. Ehrlich, *Biochem. Z.*, **101,** 276 (1920); C. Neuberg and M. Sandberg, *ibid.*, **109,** 290 (1920).

(107) N. L. Söhngen, *Chem. Weekblad*, **11,** 240 (1913); *Zentr. Bakt. Parasitenk. Abt. II*, **40,** 465 (1914).

(108) P. J. G. Mann and J. H. Quastel, *Nature*, **158,** 154 (1946).

In view of the interest attached to an explanation of the mechanism of their origin from inorganic nitrogenous material, the phytochemical reduction of nitro compounds was investigated.

Fermenting yeast is able to reduce added nitrobenzene to the corresponding amine, aniline, to quite a considerable extent. Part of the added nitrobenzene remains unattacked, but 70% of it could be converted to aniline.[109] Since a direct reduction of the nitro group to the amino group is improbable, Neuberg and Welde[110] tried phytochemical treatment of the possible intermediaries, namely, nitrosobenzene and phenylhydroxylamine on the one hand and azoxybenzene and azobenzene on the other hand.



It turned out that only the compounds listed on the right side of the scheme, namely those containing one nitrogen atom, are reduced to the aniline base by fermenting yeast; no distinct reduction of the azo derivatives was observed. Whether this would be possible by altering the experimental conditions cannot be stated offhand (see p. 100).

The experimental procedure was essentially the same for all substances mentioned, namely, the method used in the case of nitrobenzene. Some complication arises, however, due to the fact that the solids do not disperse as easily in the fermentation mixture. Although the materials were always added in alcoholic solution to the yeast preparation, partial precipitation was unavoidable. With 21.4 g. of nitrosobenzene a yield of 4 g. of aniline was obtained; addition of the same quantity of phenylhydroxylamine gave 7.5 g. of aniline. In both cases azobenzene appeared as a by-product and could be isolated in the pure state. Its formation can be readily explained by a condensation of the intermediary nitrosobenzene with the end product aniline.

It seems remarkable that phenylhydroxylamine in the concentration employed does not noticeably inhibit fermentation and nitrosobenzene causes only a transitory reduction in the rate.

(109) C. Neuberg and E. Welde, *Biochem. Z.*, **60,** 474 (1914).
(110) C. Neuberg and E. Welde, *Biochem. Z.*, **67,** 18 (1914).

The behavior of polynitro compounds has also been investigated. The results[111] obtained with *m*-dinitrobenzene will be reported since Lipschitz's[112] findings when studying this substance from other points of view have aroused interest. The latter author states that on contact with animal tissues *m*-dinitrobenzene is mainly converted to *m*-nitrophenylhydroxylamine. Although he could not obtain this compound in a pure state, he was able to identify it by its reactions. The author also mentions experiments made with yeast and with germinating seeds; an intense yellow coloration was produced; this can be related to the formation of *m*-nitrophenylhydroxylamine. The same applied to the colorimetric test with sodium carbonate solution (violet coloration). (It has, however, been stressed by Brand and Steiner[113] and also by Waterman and Kalff[114] that such color reactions are due to a contaminant of impure *m*-dinitrobenzene, and not to the hydroxylamine derivative.) Furthermore, prolonged contact with the tissue material produced a liquid which gave a distinct diazo reaction. This result points to the formation of an amine. The positive diazo reaction does not justify deductions as to the presence of *m*-nitroaniline. The transformation of *m*-dinitrobenzene can lead to a whole series of possible amino derivatives, for example, the corresponding aminonitrosobenzene, aminonitroazoxybenzene, diaminoazoxybenzene, diaminohydrazobenzene, nitroaminoazobenzene, and so forth.

It follows from the experiments of Neuberg and Reinfurth[111] that, under the conditions set up by them, *m*-nitroaniline results. The substantial difference between their method and that of Lipschitz is that they worked with fermenting sugar solutions, that is, in the presence of carbohydrates, which according to Lipschitz should not be held responsible for the reduction effect observed by him. With fermenting yeast the formation of *m*-nitroaniline from *m*-dinitrobenzene takes place within a few hours. At the end of the fermentation, during the course of which considerable quantities of *m*-nitroaniline may separate, some unchanged starting material, *m*-dinitrobenzene, is still present. A fair amount of *m*-dinitroazoxybenzene occurs as a by-product; it is formed as a secondary product of condensation of the intermediary hydroxylamino- and nitroso-derivatives or by decomposition of the *m*-nitrophenylhydroxylamine. Fundamentally, the biological reduction of 2,4,6-trinitrotoluene (TNT),

(111) C. Neuberg and E. Reinfurth, *Biochem. Z.*, **139**, 561 (1923).

(112) W. Lipschitz, *Z. physiol. Chem.*, **109**, 250 (1920); *Arch. ges. Physiol. Pflügers*, **196**, 463 (1922).

(113) K. Brand and J. Steiner, *Ber.*, **55**, 882 (1922).

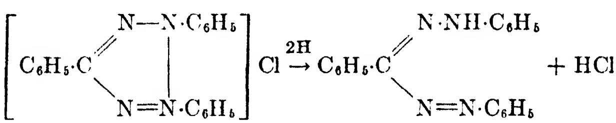
(114) N. Waterman and J. Kalff, *Biochem. Z.*, **135**, 174 (1923).

studied during recent years,[115] takes the same course. The products include 4-hydroxylamino-2,6-dinitrotoluene, 4-amino-2,6-dinitrotoluene, 6-amino-2,4-dinitrotoluene, 2-amino-4,6-dinitrotoluene and 2,4-diamino-6-nitrotoluene.

The connection of the aromatic mono-, di- and tri-nitro compounds with phytochemical reduction follows from their activating action in alcoholic fermentation, known for a quarter century.[106] The same applies equally to the excellent activating effect of cinnamic aldehyde;[106] its behavior during phytochemical reduction is described on pages 79 and 105.

Regarding the phytochemical reduction of oximes see page 101.

Phytochemical reduction of tetrazolium salts has been observed by Kuhn and Jerschel.[116] If 2,3,5-triphenyl tetrazolium chloride is added to fermenting yeast the corresponding formazan is formed. Like most formazyl compounds it has a red color.



The iron salts of the dimethyl ester of acetylhematoporphyrin and of the tetramethylhematoporphyrin yield verdoparahematin when treated with fermenting yeast under anaerobic conditions. The reduction product has been isolated by Stier[117] as a double compound with pyridine. Mention may also be made of the reduction of hydroxyhemoglobin by means of yeast, which has been investigated more closely by Neumann.[118] A few acid and basic dyestuffs are very slowly cleaved by reduction with fermenting yeast. In the case of *p*-aminoazobenzene, *p*-dimethylaminoazobenzene and 2,4-diaminoazobenzene, aniline has been claimed as the

(115) B. B. Westfall and M. I. Smith, *Proc. Soc. Exptl. Biol. Med.*, **51,** 122 (1942); H. J. Channon, G. T. Mills and R. T. Williams, *Biochem. J.*, **38,** 70 (1944); R. Lemberg and J. P. Callaghan, *Nature*, **154,** 768 (1944); *Australian J. Exptl. Biol. Med. Sci.*, **23,** 1, 6, 13 (1945); E. Bueding and N. Jolliffe, *J. Pharmacol. Exptl. Therap.*, **88,** 300 (1946).

(116) R. Kuhn and D. Jerschel, *Ber.*, **74,** 949 (1941); **77,** 591 (1944). See also H. J. Cottrell, *Nature*, **159,** 748 (1947); A. M. Mattson, C. O. Jensen and R. A. Dutcher, *Science*, **106,** 294 (1947).

(117) E. Stier, *Z. physiol. Chem.*, **275,** 155 (1942). Regarding formation of coproporphyrin in yeast preparations, see J. E. Kench and J. F. Wilkinson, *Biochem. J.*, **40,** 660 (1946).

(118) G. Neumann, *Biochem. Z.*, **281,** 181 (1935). See also H. R. Gutmann, B. J. Jandorf and O. Bodansky, *Federation Proc.*, **6,** 257 (1947); *J. Biol. Chem.*, **169,** 145 (1947). Statements regarding the mechanism of reduction of methemoglobin can be found in the paper of Q. H. Gibson, *Bioch. J.*, **42,** 13 (1948).

product.[119] Riboflavin also can undergo phytochemical reduction.[120] Reference may be made to the phytochemical reduction of the oximino group. The transformation of oximinopyruvic acid to alanine,[121] of quinone monoxime (*p*-nitrosophenol) to *p*-aminophenol[80] and of quinone dioxime to *p*-phenylenediamine[80] have been reported. Endres[122] has shown that oxime intermediates form during nitrogen assimilation. Virtanen and Laine[123] were able to convert the oxime of oxaloacetic acid into L-aspartic acid phytochemically, by means of bacteria of root nodules. The reduction of nitrate and nitrite also takes place by way of hydroxylamine.[124] Virtanen and Csáky[125] determined the formation of oxime nitrogen in torula yeast fed with KNO_3 and the conversion of the oxime into alanine and dicarboxylic amino acids or their amides. The same authors furnished the proof, important for the general problems of bioreductions, that torula grows with hydroxylamine as the sole nitrogen source. The hydroxylamine which has been found in higher plants[125a] is probably formed by an analogous mechanism.

In biosyntheses of amino acids from keto acids and ammonia, a biochemical reduction of the imino group takes place. This is the reverse process of the oxidative desamination of amino acids. Part of the keto acids clearly can be derived from carbohydrates. A review by Wieland[126] points out the relationship with phytochemical reduction.

XI. General Considerations Regarding the Character of Phytochemical Reductions

Phytochemical reduction must be regarded as a special case of oxidation-reduction. Since it is enzymatically conducted[23,94] it should be distinguished from the analogously formulated Cannizzaro reaction. For this reason Neuberg, Hirsch and Reinfurth[127] suggested the name "dismutation"[127a] for the physiological process, and in the last 25 years

(119) H. Riedel, *Klin. Wochschr.*, **21,** 569 (1942).

(120) R. J. Hickey, *Arch. Biochem.*, **11,** 265 (1946).

(121) K. Maurer, *Biochem. Z.*, **189,** 216 (1927).

(122) G. Endres, *Ann.*, **512,** 54 (1934); **518,** 109 (1935).

(123) A. I. Virtanen and T. Laine, *Suomen Kemistilehti*, **9B,** 5 (1936); *Chem. Abstracts*, **30,** 7620 (1936); *Biochem. J.*, **33,** 412 (1939).

(124) G. Endres and L. Kaufmann, *Ann.*, **535,** 1 (1938).

(125) A. I. Virtanen and T. Z. Csáky, *Nature*, **161,** 814 (1948).

(125*a*) M. Lemoigne, P. Monguillon and R. Desveaux, *Compt. rend.*, **201,** 1067, 1437 (1935).

(126) T. Wieland, *Die Chemie*, **55,** 150 (1942).

(127) C. Neuberg, J. Hirsch and E. Reinfurth, *Biochem. Z.*, **105,** 308 (1920).

(127*a*) The concept of biochemical dismutation between two different carbonyl compounds appears for the first time in an article on the processes of fermentation,

this name has become commonly employed. The disproportionation of aldehydes and hydroxy aldehydes of the aliphatic series is readily carried out enzymatically with stereochemical selectivity, but can only be achieved in exceptional cases with dilute alkali.[128] The crossed disproportionation of different aldehydes was successfully accomplished in a special purely chemical way by Nord[129] in the case of isovaleraldehyde and benzaldehyde and then by Nakai[130] and by Endoh[131] and others.[132] Oxidation-reduction between representatives of two different classes of compounds has also been realized in a precise manner by Neuberg and Gorr,[133] who obtained 2-pentanol and benzoic acid from methyl propyl ketone and benzaldehyde. Gordon[134] obtained (*levo*)-neomenthol and benzoic acid from (*levo*)-menthone and benzaldehyde. Other examples of crossed dismutations are the disproportionation between succinic acid and methylene blue (Thunberg[135]), between succinic acid and dithioglycolic acid (Wieland and Bergel[136]) and between lactate and a dyestuff, yielding pyruvic acid as well as the leuco base (Harden and Norris,[137] Aubel and Salabartan, Quastel[138]). The biochemical dismutation between two different aldehydes has been achieved by Molinari;[139] by means of *B. ascendens* he obtained more than 80% of the theoretically possible yield of ethanol and pyromucic (furoic) acid from acetaldehyde and furfural, but he did not get either acetic acid or furfuryl alcohol.

All these processes are analogous to the reduction by means of fermentation hydrogen ("Gärungswasserstoff"), namely, by reactions of the

written by C. Neuberg in C. Oppenheimer's "Handbuch der Biochemie," Ergänzungsband 1913, p. 582. Nord (ref. 129) and also Endoh (ref. 131) use the term "mixed dismutation"; Neuberg and Gorr (ref. 133) introduced "crossed dismutation."

(128) A. Lieben, *Monatsh.*, **22,** 289 (1901); H. Hammersten, *Ann.*, **420,** 262 (1920).

(129) F. F. Nord, *Biochem. Z.*, **106,** 275 (1920).

(130) R. Nakai, *Biochem. Z.*, **152,** 258 (1924).

(131) C. Endoh, *Rec. trav. chim.*, **44,** 866 (1925).

(132) N. A. Orloff, *Bull. soc. chim.*, [4] **35,** 360 (1924); J. A. Pearl, *J. Org. Chem.*, **12,** 79, 85 (1947).

(133) C. Neuberg and G. Gorr, *Biochem. Z.*, **166,** 444 (1925).

(134) M. S. Gordon, *J. Biol. Chem.*, **75,** 163 (1927). See also D. Davidson and M. T. Bogert, *J. Am. Chem. Soc.*, **57,** 905 (1935); C. D. Nenitzescu and I. Gavăt, *Bul. soc. chim. România*, **16A,** 42 (1934); *Chem. Abstracts*, **30,** 5572 (1936); H. E. French and D. M. Gallagher, *J. Am. Chem. Soc.*, **64,** 497 (1942).

(135) T. Thunberg, *Skand. Arch. Physiol.*, **35,** 163 (1917); **40,** 1 (1920).

(136) H. Wieland and F. Bergel, *Ann.*, **439,** 204 (1929).

(137) A. Harden and D. Norris, *Biochem. J.*, **8,** 100 (1914); **9,** 330 (1915).

(138) E. Aubel and J. Salabartan, *Compt. rend.*, **180,** 1183, 1784 (1925); J. H. Quastel, *J. Hyg.*, **28,** 139 (1928).

(139) E. Molinari, *Biochem. Z.*, **216,** 187 (1929).

labile dihydrocozymase with the substance which is to be phytochemically reduced.

The effect of phytoreduction of different types of substances is essentially a result of a competition between the added hydrogen acceptor and the natural acceptor, acetaldehyde. When the latter is displaced it can be identified as such or in the form of its products of dismutation or carboligatic synthesis (acyloin formation).

The transfer of hydrogen in the course of glycolytic processes is effected by cozymase (codehydrase I). The reactive group of cozymase is a nicotinamide residue which acts as acceptor for the "Gärungswasserstoff" (see p. 76). The resulting labile dihydro compound then transmits the loosely bound hydrogen to the acceptors. In this way the oxidation-reduction process is kept going. The fact that apozymase[140] lacks the ability to initiate reductions establishes that dismutation requires the presence of a coenzyme. Harden and Norris,[137] as well as von Euler and Nilsson,[141] proved the necessity of coenzyme for the reduction of methylene blue, Myrbäck and Jacobi,[142] von Euler and Myrbäck[143] and von Euler, Grabe and Adler[144] for the reduction of acetaldehyde, and Neuberg and Kobel[145] for the intramolecular oxidoreduction that takes place when methylglyoxal is converted to lactic acid. Szent-Györgyi and associates[146] showed that cozymase is essential for the reduction of oxalacetic acid.

Warburg's yellow enzyme mediates the transfer of hydrogen from the donors to cozymase (codehydrase). Of the substances taking part in the alcoholic cleavage of sugar, ethanol and the Neuberg ester (D-fructose-6-phosphate) can act, according to Fischer and Eysenbach,[90] as special donors. These authors ascertained that in the biological reduction of fumaric acid to succinic acid the leucoflavin enzyme is also the hydrogen donor. Codehydrase reduces the yellow enzyme. The leucoflavin (see also von Euler and Adler[144]) transfers the hydrogen to the acceptor, but

(140) A. Harden and Marjorie G. Macfarlane, *Biochem. J.*, **25**, 818 (1931).

(141) H. von Euler and R. Nilsson, *Z. physiol. Chem.*, **149**, 44 (1925); **160**, 234 (1926); **162**, 72, 264 (1927).

(142) K. Myrbäck and W. Jacobi, *Z. physiol. Chem.*, **161**, 245 (1926).

(143) H. von Euler and K. Myrbäck, *Z. physiol. Chem.*, **165**, 28 (1927).

(144) H. von Euler and Elsa Grabe, *Arkiv Kemi, Mineral. Geol.*, **9**, 1 (1928); *Chem. Centr.*, **1928 I**, 2410; H. von Euler and E. Adler, *Z. physiol. Chem.*, **238**, 233 (1936).

(145) C. Neuberg and Maria Kobel, *Biochem. Z.*, **207**, 232 (1929); also older literature quoted there.

(146) E. Annau, I. Banga, B. Gözsy, S. Huszák, K. Laki, B. Straub and A. Szent-Györgyi, *Z. physiol. Chem.*, **236**, 1 (1935).

may also be reoxidized slowly by autoreduction or faster by cytochrome C as postulated by Theorell.[147] As a result of the increased knowledge, Fischer and Eysenbach[88,90] designated the cozymase-flavin enzyme combination as the "Gärungswasserstoff" system. They also considered triose phosphate as a hydrogen donor. Gottschalk[148] proposed that dihydrocozymase, formed from cozymase during the oxidation of glyceraldehyde phosphate to phosphoglyceric acid (Warburg), gives up its hydrogen to the phytochemically reducible substrate instead of to its natural acceptor, acetaldehyde, which is displaced in this way. Illustration of this procedure is given by the example of diacetyl. Gottschalk, confirming previous statements (see p. 86), has established acetylmethylcarbinol and 2,3-butanediol as reduction products of diacetyl and has reported the rate of reduction in the second stage to be twice that of the first stage. In the same publication the author announced that in an atmosphere of nitrogen, alcoholic fermentation and total carbohydrate consumption are inhibited by the addition of acetaldehyde. He considered this behavior to be a counterpart of the Pasteur reaction in yeast (see below). The sluggish reduction of molecular oxygen by dihydrocozymase, mediated through the flavoprotein system, offers to other reducible substances the opportunity to compete with oxygen in obtaining the "Gärungswasserstoff" from dihydrocozymase. This behavior indicates to the author that there is a relationship between phytochemical reduction and the Pasteur effect under anaerobic conditions. Entirely different explanations have been given for this phenomenon by others, for example, by Hoogerheide,[149] Lipmann,[150] Johnson,[151] Engelhardt and Sakov,[152] Fink and coworkers,[153] and Colowick and Price.[154]

The reduction of aromatic nitro compounds (see p. 98) is also due to the action of an enzyme system in which a dehydrogenase transfers hydrogen to a diphosphopyridine nucleotide-flavoprotein, which in turn reduces the nitro group (Westfall,[155] as well as Bueding and Jolliffe[11b]).

The following results likewise indicate the relationship between phyto-

(147) H. Theorell, *Nature*, **138**, 687 (1936).

(148) A. Gottschalk, *Australian J. Exptl. Biol. Med. Sci.*, **19**, 211 (1941); **20**, 173 (1942).

(149) J. C. Hoogerheide, Thesis, Delft, (1935).

(150) F. Lipmann, "Symposium on Respiratory Enzymes," Univ. Wisconsin Press, Madison, p. 48 (1942).

(151) J. Johnson, *Science*, **94**, 200 (1941).

(152) A. V. Engelhardt and N. E. Sakov, *Biokhimiya*, **8**, 9 (1943). See also D. Burk and R. J. Winzler, *Ann. Rev. Biochem.*, **XIII**, 497 (1944).

(153) H. Fink, J. Krebs and R. Lechner, *Biochem. Z.*, **301**, 137, 143 (1939).

(154) S. P. Colowick and W. H. Price, *J. Biol. Chem.*, **157**, 415 (1945).

(155) B. B. Westfall, *J. Pharmacol. Exptl. Therap.*, **78**, 386 (1943).

chemical reduction and the process of alcoholic fermentation. In 1915 Neuberg[156] and Oppenheimer[157] ascertained that cell-free fermentation of D-glucose and D-mannose is greatly stimulated by the addition of acetaldehyde or pyruvic acid. All the higher α- keto acids cleavable by carboxylase behave in the same way.[158] It has further been found[159] that many reducible substances, both organic and inorganic and 76 in number, show essentially the same effect of stimulation. This applies to living yeasts too, but the stimulation is far more marked in cell-free systems. The results that were obtained have been explained through analogy with the pyruvic acid-acetaldehyde theory of fermentation put forward a few years previously[1] (see also later reference[230]). Due to the ability of the stimulators to be reduced, they can act as acceptors for the hydrogen which is to be taken up by some suitable substance during the step in which pyruvic acid is formed (see p. 76). In fact, the added activator disappears in the course of fermentation. Since in normal alcoholic sugar cleavage a constant low level of acetaldehyde is maintained, this condition being necessary for the continuance of the process,[160] it can easily be imagined that at the start the requisite concentration is not yet attained and has to be produced by the act of fermentation itself. As mentioned previously (see p. 82), acetaldehyde may be considered as the primary equivalent of a product of phytochemical reduction, so that the effect of added stimulators of this type is understandable. (It should be remembered that the activating mixture usually employed to overcome the so-called induction period always contains an aldehyde beside traces of manganese dichloride, magnesium chloride and hexose diphosphate.[161]) Harden and Henley[162] have confirmed these findings[160] and extended them, stating that the phytochemically reducible activators favor the more rapid start of phosphorylation. Experiments regarding the nature of this remarkable stimulating effect have been carried out by Harden and Henley in order to ascertain which of the processes occurring in yeast juice are chiefly concerned. Harden writes:[163] "It is thus seen why the Neuberg effect is always greater towards the begin-

(156) C. Neuberg, *Biochem. Z.*, **71**, 1 (1915).

(157) M. Oppenheimer, *Z. physiol. Chem.*, **93**, 235 (1915).

(158) C. Neuberg and E. Schwenk, *Biochem. Z.*, **71**, 135 (1914).

(159) C. Neuberg, *Biochem. Z.*, **88**, 145 (1918); C. Neuberg and Marta Ehrlich, *ibid.*, **101**, 239, 276 (1920); C. Neuberg, Elsa Reinfurth and Marta Sandberg, *ibid.*, **121**, 215 (1921).

(160) C. Neuberg and J. Kerb, *Ber.*, **47**, 2730 (1914); C. Neuberg and E. Schwenk, *Biochem. Z.*, **71**, 126 (1915); C. Neuberg and J. Hirsch, *ibid.*, **100**, 304 (1919).

(161) C. Neuberg and H. Lustig, *Arch. Biochem.*, **1**, 194, 317 (1942).

(162) A. Harden and F. R. Henley, *Biochem. J.*, **14**, 642 (1920), **15**, 175 (1921).

(163) A. Harden, "Alcoholic Fermentation," Longmans, Green and Co., London, 4th ed., p. 150 (1932).

ning of fermentation when the phosphate is still present as mineral phosphate. Neuberg's theory of the action of activators can be applied almost without modification to these results."

The long-known stimulating effect of mono- and polynitro compounds[106,159] on the onset of fermentation in yeast maceration juice has been reinvestigated by Vandendriessche.[164] The induction time is shortened significantly by 2,4- or 2,5-dinitrophenol, while 2,6-dinitrophenol did not show such an effect. The influence is evident when using as substrates the fermentable hexoses and D-fructose-6-phosphate, but not hexose diphosphate. According to Markovičev[165] a stimulation of the oxidation processes can be proved thereby. It is probable that these effects are related to the known phytochemical reduction of nitro compounds (see pp. 98 and 99).

XII. Phytochemical Reductions by Means of Microbes

The previously discussed reductions by yeast of isovaleraldehyde to isoamyl alcohol, of *p*-xyloquinone to *p*-xylohydroquinone and of sodium thiosulfate to hydrogen sulfide have also been accomplished with *Bacterium coli* and *Bacterium lactis aerogenes*.[166] Phytochemical reduction of d,l-valeraldehyde (methylethylacetaldehyde) with *Termobacterium mobile* Lindner (*Pseudomonas Lindneri*) takes a practically quantitative course and yields an amyl alcohol containing 17% excess of the dextrorotatory component.[167] Cahill[168] achieved especially favorable results by the reduction of isovaleraldehyde with growing bacteria instead of with their mass cultures.

By means of *B. coli* crotyl alcohol (2-butene-1-ol) can be slowly reduced phytochemically to *n*-butanol.[169] The same bacterium does not attack cinnamyl alcohol,[169] but does slowly convert dehydrocholic acid to 7-hydroxy-3,12-diketocholic acid.[170]

Diacetyl as well as acetylmethylcarbinol are reduced to 2,3-butylene glycol (see p. 86) by lactic acid bacilli and streptococci.[171]

(164) L. Vandendriessche, *Enzymologia*, **10**, 69 (1941); L. Massart and L. Vandendriessche, *ibid.*, **10**, 244 (1942).

(165) L. Markovičev, *Ber. Kgl. Serb. Acad., Ser. I*, **180**, 275 (1939); *Chem. Abstracts*, **35**, 7109 (1941).

(166) C. Neuberg and E. Simon, *Biochem. Z.*, **190**, 226 (1927).

(167) S. Forssman, *Biochem. Z.*, **264**, 228 (1933).

(168) W. M. Cahill, *Fermentforschung*, **15**, 134 (1936).

(169) F. G. Fischer and W. Robertson, *Ann.*, **529**, 85 (1937).

(170) F. Fukui, *J. Biochem. Japan*, **25**, 61 (1937).

(171) A. J. Kluyver, *J. Soc. Chem. Ind. London*, **52**, 367 (1933); B. W. Hammer, G. L. Stahly, C. H. Werkman and M. B. Michaelian, *Iowa Agr. Expt. Sta. Research Bull.*, **191**, 381 (1935); G. G. Freeman, *Biochem. J.*, **41**, 389 (1947).

In a somewhat ambiguous manner crotonbetaine, $(CH_3)_3 \cdot N^+ \cdot CH_2 \cdot$-$CH{=}CH \cdot COO^-$, and carnitin, $(CH_3)_3 \cdot N^+ \cdot CH_2 \cdot CHOH \cdot CH_2 \cdot COO^-$, were hydrogenated to γ-butyrobetaine, $(CH_3)_3 \cdot N^+ \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot COO^-$, by undefined mixtures of bacteria of putrefying pancreas in the presence of D-glucose after the long time of four months.[172]

In the steroid series phytochemical reductions also have been accomplished by means of bacteria, not always with pure cultures.[173]

Hickey[120] observed an interesting reduction of riboflavin by *Streptococcus faecalis* to green and reddish orange products, which may be related to the well-known verdo- and rhodo-flavin.

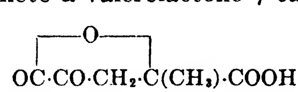
Both *B. coli* and *B. lactis aerogenes* belong to a class of organisms that are able to develop free hydrogen under certain conditions. Their metabolism thus shows certain phases approaching those phenomena of other bacteriological fermentations in which the formation of hydrogen is a necessary step.

More than seventy years ago the impressive discovery was made that bioreduction of mannitol, glycerol and starch yields butanol.[174]

Fermentations in which butyric acid, butanol and acetone are formed from carbohydrates by different bacilli (butyl bacteria) belong in this group. The term butyl bacteria as a generic name for microbes producing the genetically related substances of the four-carbon series was proposed in 1921[175] and has been applied since then. The approximate course of these reactions is shown by the following formulations which, however, do not explain the mechanism:

$$C_6H_{12}O_6 \rightarrow 2\,CO_2 + 4\,H + CH_3 \cdot CH_2 \cdot CH_2 \cdot COOH$$
$$C_6H_{12}O_6 \rightarrow 2\,CO_2 + H_2O + CH_3 \cdot CH_2 \cdot CH_2 \cdot CH_2OH$$
$$C_6H_{12}O_6 + H_2O \rightarrow 3\,CO_2 + 8\,H + CH_3 \cdot CO \cdot CH_3.$$

According to an early report by Neuberg and Arinstein,[175] the conversion of carbohydrates to butyric acid and higher homologs does not take place directly at the pyruvic acid stage, which occupies a central position during degradation and transformation of carbohydrates, but through the lactone of the pyruvic acid aldol, $HOOC \cdot CO \cdot CH_2 \cdot C(OH)$-$(CH_3) \cdot COOH$, the α-keto-α-valerolactone-γ-carboxylic acid. Peldán,



(172) W. Linneweh, *Z. physiol. Chem.*, **181,** 54 (1929).

(173) A. Ercoli, *Ber.*, **71,** 156, 650 (1938); L. Mamoli and G. Schramm, *ibid.*, **71,** 1322, 2088, 2698 (1938); L. Mamoli, R. Koch and H. Tescher, *Z. physiol. Chem.*, **261,** 287 (1939).

(174) References quoted in footnote 198.

(175) C. Neuberg and B. Arinstein, *Biochem. Z.*, **117,** 269 (1921). See also H. Suomalainen and E. Arhimo, *ibid.*, **317,** 59 (1943).

van der Lek and Virtanen and Sundman[176] confirmed this mechanism by achieving a practically quantitative fermentation of pyruvic acid aldol anhydride to butyric acid.

The formation of butyl alcohol by the reduction of butyric acid, or other acids which can be converted to butyric acid, is a biochemical process of the utmost general importance. It involves the biochemical reduction of a carboxyl group. Obtained as early as 1920, these findings never have been given due consideration. Speakman[177] determined that butyric acid is converted into butanol to the extent of 80%. It was accompanied by 10% acetone. The same result was obtained by Reilly, Hickinbottom, Henley and Thaysen.[178] A few years later Bakonyi[179] showed that the analogous reduction of acetic acid is practically quantitative; Johnson, Peterson and Fred[180] demonstrated the process in the case of pyruvic acid, an intermediate in the reduction of sugar to butyric acid. Blanchard and MacDonald[181] succeeded in reducing biochemically propionaldehyde and propionic acid to *n*-propyl alcohol with microorganisms of the butyl fermentation group, and Bernhauer and Kürschner[182] were able to convert crotonic acid (2-butenoic acid) to *n*-butanol. Simon and Weizmann[183] established the reduction of propionic and butyric acid by means of *Clostridium acetobutylicum.*

The reduction of butyric acid seems to proceed by way of *n*-butyraldehyde. The phytochemical reduction to butyl alcohol previously demonstrated for yeast (see p. 78) has also been carried out with bacteria.[182] Regarding the reduction of butyric acid to *n*-butanol, papers of Bernhauer and coworkers,[184] Kluyver and Donker,[185] Stiles, Peterson and Fred,[186] Janke and Siedler[187] and Wood, Brown and Werkman[188] should be consulted. Peynaud[189] ascribes also to wine yeast the ability to

(176) H. Peldán, *Biochem. Z.*, **309,** 131, 143 (1941); J. B. van der Lek, Dissertation, Delft, (1930); A. I. Virtanen and I. Sundman, *Biochem. Z.*, **313,** 237 (1942).

(177) H. B. Speakman, *J. Biol. Chem.*, **41,** 319 (1920); **58,** 395 (1923).

(178) J. Reilly, W. J. Hickinbottom, F. R. Henley and A. C. Thaysen, *Biochem. J.*, **14,** 229 (1920).

(179) S. Bakonyi, *Biochem. Z.*, **169,** 125 (1926).

(180) M. J. Johnson, W. H. Peterson and E. B. Fred, *J. Biol. Chem.*, **101,** 145 (1933).

(181) K. C. Blanchard and J. MacDonald, *J. Biol. Chem.*, **110,** 145 (1935).

(182) K. Bernhauer and K. Kürschner, *Biochem. Z.*, **280,** 379 (1935).

(183) E. Simon and C. Weizmann, *Enzymologia*, **4,** 169 (1937).

(184) K. Bernhauer, B. Görlich and E. Köcher, *Biochem. Z.*, **287,** 61 (1936).

(185) A. J. Kluyver and H. J. L. Donker, *Chem. Zelle u. Gewebe*, **13,** 134 (1926).

(186) H. R. Stiles, W. H. Peterson and E. B. Fred, *J. Biol. Chem.*, **84,** 437 (1929).

(187) A. Janke and V. Siedler, *Biochem. Z.*, **292,** 101 (1937), **293,** 453 (1937).

(188) H. G. Wood, R. W. Brown and C. H. Werkman, *Arch. Biochem.*, **6,** 243 (1945).

(189) E. Peynaud, *Ann. fermentations*, **5,** 321, 385 (1939/1940).

reduce volatile fatty acids to the corresponding alcohols. The oldest observation in this respect appears to be due to Söhngen,[190] who reported that acetic acid bacteria are capable of reducing acetic acid to ethanol, although to only a small extent. Today these reductions of carboxyl groups to alcohol groups, discovered more than a quarter of a century ago, can be readily understood. Warburg, Christian, Negelein and Brömel[191] ascertained that, in the course of the oxidation-reductions taking place during glycolytic processes, glyceric acid, phosphorylated at the carboxyl group, is converted to phosphorylated glyceraldehyde. This fundamental discovery represents a biochemical reduction of the carboxyl group. Just as this process depends on the active participation of codehydrase, coupled phosphorylation may play a part in butanol fermentation.

In 1904, Schardinger[192] discovered the bacteriological formation of acetone from carbohydrates, and Pringsheim,[193] in the years 1905–1909, described the reduction of carbohydrates to isopropyl alcohol and *n*-butyl alcohol. The subsequent work of Fernbach[194] and Weizmann[195] led to the development of an industry for the production of these substances by the fermentation of carbohydrates.[196]

A new species of the *Acetobutylicum* group is described by Owen, Mobley and Arroyo.[197] This bacillus, *Clostridium tetrylium*, obtained from soil surrounding the roots of the sugar cane in Puerto Rico, produces 75% butanol, 20% acetone, 5% ethanol and no isopropanol at all, thus differing from other butyl bacteria.

Numerous investigations concerning the mechanism of these reductive fermentations have been reported. Basically it is important that pentoses (L-arabinose and D-xylose) yield the same products of fermenta-

(190) N. L. Söhngen, *Folia microbiol.*, **3**, 151 (1914); *Chem. Centr.*, *I*, 326 (1915).

(191) O. Warburg and W. Christian, *Biochem. Z.*, **303**, 40 (1939); E. Negelein and H. Brömel, *ibid.*, **301**, 135 (1939).

(192) F. Schardinger, *Wien. Klin. Wochschr.*, **17**, 207 (1904); *Zentr. Bakt. Parasitenk., Abt. II*, **14**, 772 (1905); **29**, 188 (1911).

(193) H. Pringsheim, *Zentr. Bakt. Parasitenk., Abt. II*, **15**, 319 (1905); **20**, 248 (1908), *Biochem. Z.*, **10**, 490 (1908); **16**, 243 (1909).

(194) A. Fernbach and E. H. Strange, British Pat. 21073 (1912).

(195) C. Weizmann, British Pat. 4845 (1915), quoted in *Chem. Centr.*, *II*, 34 (1921).

(196) Concerning the history of butanol-acetone fermentation see: C. F. Arzberger, W. H. Peterson and E. B. Fred, *J. Biol. Chem.*, **44**, 465 (1920); C. L. Gabriel, *Ind. Eng. Chem.*, **20**, 1063 (1929); A. Jørgensen, "Microorganisms and Fermentations." Lippincott, Philadelphia, 6th ed. rewritten by Hansen, Lund and Mitchell, p. 365 (1939); S. C. Prescott and C. G. Dunn, "Industrial Microbiology," McGraw-Hill Co., New York, p. 183 (1940).

(197) W. L. Owen, R. L. Mobly and R. Arroyo, *Zentr. Bakt. Parasitenk., Abt. II*, **95**, 131 (1936).

tion as hexoses.[176,197] The quantitative ratio of the final products of fermentation does not differ very much for hexoses and pentoses. Van der Lek[176] stipulates glycolaldehyde as an intermediary cleavage product. According to Mull and Nord[197a] this intermediary plays a part in *Fusarium* fermentations.

Important results have recently been obtained by Simon.[197b] Among other things he ascertained that glycerol yields butyric acid. The formation of four-carbon compounds from six-carbon substrates is independent of the grouping (aldehyde, hydroxyl, carboxyl, phosphorylated hydroxyl) at the first carbon atom of the molecule. L-Rhamnose and D-arabitol are fermented, but not D-arabinose and D-sorbitol. In contrast to the studies of Underkofler and Hunter,[197c] L-sorbose has been found fermentable. Results obtained with fresh and acetone-dried *Cl. butylicum* are identical in principle.

Reductive fermentations can be classified into the following types: (*1*) butyric acid fermentation, (*2*) butanol-acetone fermentation, (*3*) butanol-isopropyl alcohol fermentation, (*4*) butylene glycol-ethanol fermentation, and (*5*) acetone-ethanol fermentation.

Differentiation has been based on the main products of the fermentation. Generally, fermentation does not yield homogeneous products. For example, butyric acid is practically always accompanied by other fatty acids, both lower and higher in the series, as well as by alcohols, especially butanol, and by lactic acid.[198] It may be noted that, according to Kempner and Kubowitz,[199] fermentation can be transformed into lactic acid fermentation if the fermentation is carried out in an atmosphere of carbon monoxide.

During butanol-acetone fermentation, acetylmethylcarbinol and 2,3-butylene glycol[200] nearly always arise; ethanol and isopropyl alcohol occur and methyl ethyl ketone has also been observed as a by-product.

Intermediate products which have been identified by isolation or inferred as precursors of butyric acid, butyl alcohol, acetone and isopropyl alcohol are: acetaldehyde, acetaldol, pyruvic acid and its aldol,

(197*a*) R. P. Mull and F. F. Nord, *Arch. Biochem.*, **5**, 283 (1944).

(197*b*) E. Simon, *Arch. Biochem.*, **14**, 39 (1947).

(197*c*) L. A. Underkofler and J. E. Hunter, Jr., *Ind. Eng. Chem.*, **30**, 480 (1938).

(198) V. Meyer and P. Jacobson, "Lehrbuch der organischen Chemie," Veit and Co., Leipzig, Band 1, Teil 1, p. 236 and 530 (1907).

(199) W. Kempner, *Biochem. Z.*, **257**, 41 (1933); W. Kempner and F. Kubowitz, *ibid.*, **265**, 245 (1933); F. Kubowitz, *ibid.*, **274**, 285 (1934). Concerning the influence of culture conditions, see also E. Aubel, A. J. Rosenberg and M. Gruenberg, *Helv. Chim. Acta*, **29**, 1267 (1946).

(200) J. Yamasaki and T. Karasima, *Enzymologia*, **3**, 271 (1937); R. W. Brown, G. L. Stahly and C. H. Werkman, *Iowa State Coll. J. Sci.*, **12**, 245 (1938).

acetic acid, crotonic acid, *n*-butyric acid and *n*-butyraldehyde. During the conversion of butyric acid to acetone by the group of bacteria known as symbionts, the occurrence of acetaldehyde has also been reported.[201] The formic acid which frequently has been found with acetic acid and its homologs may have been formed by hydroclastic cleavage of pyruvic acid and other keto acids according to the reaction $R{\cdot}CO{\cdot}COOH + H_2O = R{\cdot}COOH + H{\cdot}COOH$, first observed as a bacterial process as early as 1914.[202]

A critical survey of the voluminous relevant literature has been written by Kluyver.[203] Among more recent investigations are those of Ward, Pettijohn, Neish, Lockwood and Coghill and of Ledingham and associates[204] concerning butylene glycol fermentation, in which the older industrial process of Fulmer, Christensen and Kendall[205] has been improved.

In propionic acid fermentations the starting materials are a sugar and the lactic and pyruvic acids that are derived from it.[206] According to Barker and Lipmann[207] pyruvate may be reduced to propionate without passing through lactate. Propionic acid itself can be reduced to *n*-propanol.[181]

In various types of bacteriological cellulose fermentations, large-scale reduction of sugar takes place. Relevant facts as well as the older literature can be found in an article by Neuberg and Cohen,[208] while the metabolism of wood-destroying fungi is treated by Nord and Sciarini.[209] Butyric acid and other products of reduction probably are formed as in butyl fermentations. Definite results with pure cultures are hardly available[210] from most of the early papers.

The far-reaching reduction to methane is of special interest. Methane

(201) H. Bierry and P. Portier, *Compt. rend.*, **166,** 1055 (1918).

(202) C. Neuberg, *Biochem. Z.*, **67,** 90, 122 (1914); C. Neuberg and B. Rewald, *ibid.*, **71,** 123 (1915).

(203) A. J. Kluyver, *Ergeb. Enzymforsch.*, **4,** 264 (1935).

(204) G. E. Ward, O. G. Pettijohn, L. B. Lockwood and R. D. Coghill, *J. Am. Chem. Soc.*, **66,** 541 (1944); G. A. Ledingham, G. A. Adams and R. Y. Stanier, *Can. J. Research*, **23F,** 48 (1945); A. C. Neish, *ibid.*, **23B,** 10 (1945); G. A. Adams, *ibid.*, **24F,** 1 (1946); D. Rose, *ibid.*, **24F,** 320 (1946); **25F,** 273 (1947); A. C. Blackwood and G. A. Ledingham, *ibid.*, **25F,** 180 (1947).

(205) E. J. Fulmer, L. M. Christensen and A. R. Kendall, *Ind. Eng. Chem.*, **25,** 798 (1933).

(206) K. Maurer, *Biochem. Z.*, **191,** 83 (1927).

(207) H. A. Barker and F. Lipmann, *Arch. Biochem.*, **4,** 361 (1944).

(208) C. Neuberg and C. Cohen, *Biochem. Z.*, **122,** 204 (1921).

(209) F. F. Nord and L. J. Sciarini, *Arch. Biochem.*, **9,** 419 (1946).

(210) H. Pringsheim, "Die Polysaccharide," Verlag Julius Springer, Berlin, 3rd ed., p. 142 (1931).

fermentation as formulated in the older textbooks, $C_6H_{10}O_5 + H_2O = 3\,CO_2 + 3\,CH_4$, could never be verified experimentally. Some essential references can be found in papers by Barker.[211] This author ascertained the basically important fact that carbon dioxide plays an active part in the process of reduction. He demonstrated that ethanol yields methane according to the equation $2\,CH_3{\cdot}CH_2OH + CO_2 = 2\,CH_3{\cdot}COOH + CH_4$. The acetic acid that is produced probably undergoes further transformation as formulated in the scheme: $CH_3{\cdot}COOH + CO_2 + 2\,H_2O = 2\,CO_2 + 2\,H_2O + CH_4$. A similar mechanism applies to the conversion of butyl alcohol and butyric acid to acetic acid and methane. According to Barker[211] the transformation of alcohol through acetaldehyde into butyric acid, caproic acid, acetic acid and methane requires the active participation of carbon dioxide. This work establishes an analogy or relationship to the findings of Wood and Werkman[212] in which the formation of succinic acid from three-carbon compounds by the addition of carbon dioxide is demonstrated.

Fundamentally new insights have recently been gained by Kluyver and Schnellen.[212a] Making use of a pure culture of methane bacteria, *Methanosarcina Barkerii*, they converted a mixture of carbon monoxide and hydrogen into methane according to the equation: $CO + 3\,H_2 = H_2O + CH_4$. This fermentation process actually takes place in two steps: (*a*) $CO + H_2O = H_2 + CO_2$ and (*b*) $CO_2 + 4\,H_2 = 2\,H_2O + CH_4$. Many details and references to the older literature can be found in this publication and also in the thesis of Schnellen.[212b]

Two factors throw some light on the formation of reaction products of differing nature and quantitative ratio, by similar microorganisms or even by the same microbes under slightly altered conditions. One is the considerable influence that is exerted by the hydrogen ion concentration,[175] as already established in the fermentation of yeast. The other is the possibility of altering the enzymatic system by slight interference, such as dilution or the withdrawal or addition of coferments; such alterations may completely change the course of the fermentation. This subject has been studied in researches by Kubowitz,[199] Simon,[213] E. Auhagen and Neuberg,[214] E. Auhagen and T. Auhagen,[215] and Cattaneo and Neuberg.[216]

(211) H. A. Barker, *Arch. Mikrobiol.*, **7**, 404, 420 (1936); **8**, 415 (1937).

(212) H. G. Wood and C. H. Werkman, *Biochem. J.*, **30**, 48 (1936); **32**, 1262 (1938). See also A. J. Virtanen, *Nature*, 795 (1946).

(212*a*) A. J. Kluyver and C. G. T. P. Schnellen, *Arch. Biochem.*, **14**, 57 (1947).

(212*b*) C. G. T. P. Schnellen, Thesis, Delft (1947).

(213) E. Simon, *Biochem. Z.*, **224**, 253 (1930); *Arch. Biochem.*, **13**, 237 (1947).

(214) E. Auhagen and C. Neuberg, *Biochem. Z.*, **264**, 452 (1933).

(215) E. Auhagen and T. Auhagen, *Biochem. Z.*, **268**, 247 (1934).

(216) C. Cattaneo and C. Neuberg, *Biochem. Z.*, **272**, 441 (1934).

Sugar is transformed and reduced to glycerol during fermentation with *Lactobacillus mannitopoeus* or *Lactobacillus lycopersici* (heterofermentative lactic acid bacteria). This conversion, which has been described by different investigators,[217] may be formulated as follows:

$$3\,C_6H_{12}O_6 + 2\,H_2O \rightarrow 2\,CO_2 + 2\,CH_3{\cdot}COOH + 4\,CH_2OH{\cdot}CHOH{\cdot}CH_2OH$$

Beside acetic acid, there are formed ethanol, glycerol, lactic acid and sometimes even free hydrogen. The course of the reaction is complicated. In a way it may be regarded as a mixed lactic acid fermentation with a cleavage of the sugar in the sense of the third form of fermentation. Reductive cleavage of D-glucose with formation of glycerol[218] has also been achieved with *Bacterium coli* (Fernández and Garméndia) and with different kinds of fungi (Raistrick and associates). In the latter case mannitol occurs at the same time. When *Bacterium subtilis* is used (Neish and coworkers) the glycerol is accompanied by considerable quantities of 2,3-butylene glycol. Furthermore, lactic acid, volatile fatty acids and ethanol are formed.

Conditions are slightly different during the so-called mannitol fermentation of sugars, especially of D-fructose. The process, which has been known for a long time, has recently been investigated more thoroughly by Bolcato.[219] He found that 3 moles of D-fructose yield 2 moles of carbon dioxide, 2 moles of acetic acid and 2 moles of D-mannitol. Up to now, a maximum of 60% of the theoretically possible amount of D-mannitol has been isolated. The mechanism of the reaction may be assumed to be as follows:

$$C_6H_{12}O_6 = 2\,CO_2 + 4\,H + 2\,CH_3{\cdot}COOH \qquad (a)$$
$$2\,C_6H_{12}O_6 + 4\,H = 2\,C_6H_{14}O_6. \qquad (b)$$

In any case acetic acid does occur, but in addition to it lactic acid and also some ethanol and acetaldehyde may be present. Schoen and Eras[220] report that aldohexoses do not yield D-mannitol, while in addition

(217) E. B. Fred and W. H. Peterson, *J. Biol. Chem.*, **41**, 431 (1920); E. B. Fred, W. H. Peterson and Audrey Davenport, *ibid.*, **42**, 175 (1920); W. H. Peterson, E. B. Fred and J. A. Anderson, *ibid.*, **42**, 273 (1920); **48**, 385 (1921); H. R. Stiles, W. H. Peterson and E. B. Fred, *ibid.*, **64**, 643 (1925); M. E. Nelson and C. H. Werkman, *J. Bact.*, **30**, 547 (1935).

(218) O. Fernández and T. Garméndia, *Anales soc. españ. fís. quím.*, **21**, 481 (1923); *Chem. Centr.*, (1924, I); 1813; H. Raistrick and W. Rintoul, *Trans. Roy. Soc.* (*London*), **220B**, 1 (1931) and accompanying articles; A. C. Neish, A. C. Blackwood, W. E. Brown and G. A. Ledingham, *Can. J. Research*, **25B**, 56 (1947). See also U. Gayon and E. Dubourg, *Ann. inst. Pasteur*, **8**, 108 (1894), **15**, 527 (1901).

(219) V. Bolcato, *Ann. chim. applicata*, **23**, 405 (1933); *Enzymologia*, **5**, 52 (1938).

(220) M. Schoen and E. Eras, *Enzymologia*, **4**, 198 (1937). See also J. Yamasaki and M. Simonura, *Biochem. Z.*, **291**, 340 (1937).

to D-fructose, D-sorbose after rearrangement does yield it. (But see Raistrick and associates[218] regarding D-glucose.)

Topinambur tubers (*Helianthus tuberosus*, L.), which contain the D-fructose polysaccharide inulin, can undergo a mannitol fermentation through microorganisms which adhere to the nodules.[221] Similar observations have been made with "clamped" beets (that is, beets which have been conserved by covering with earth), the sucrose of which can become inverted and partially reduced to D-mannitol.[222]

According to Fink and Just,[223] yeast contains dulcitol when it has been grown in wood sugar solutions. The dulcitol may be formed by reduction from D-galactose.

In the course of processes of fermentation, bacteria are able to reduce glycerol to trimethylene glycol, $CH_2OH{\cdot}CH_2{\cdot}CH_2OH$, and 1,2-propylene glycol, $CH_3{\cdot}CHOH{\cdot}CH_2OH$. Bacteriological reductions of glycerol have been known since Freund's[224] findings and have been observed in several instances. Trimethylene glycol can contaminate the glycerol formed during glycerol fermentation of sugar by yeast.[225] Tap water contains a bacillus that is capable of reducing glycerol to trimethylene glycol.[226] More recent investigations regarding the formation of trimethylene glycol have been made by Werkman and Gillen,[227] while those regarding propylene glycol are due to Schutt.[228]

The enormous field of the reduction of sugars to fat will only be touched as it has been reviewed recently by Deuel and Morehouse.[229] This is a basic problem of the metabolism of animal and plant cells. New insight has been gained through the study of the synthesis of fatty acids by yeasts, fat molds and bacilli. Pyruvic acid and acetaldehyde were recognized as products of biochemical sugar cleavage as early as 1911, while it has been known since 1921 that they play a vital part in biosynthesis (carboligatic reaction, and others). In the same year the observation mentioned on page 107 was made, that under the influence

(221) H. H. Schlubach and H. Knoop, *Ann.*, **497**, 208 (1932).

(222) J. Vondrák, *Z. Zuckerind. čechoslovak, Rep.*, **57**, 317 (1933); *Chem. Centr.* (1933, II), 600; *Chimie & Industrie*, **29**, 1143 (1933). See also G. J. Hurker and C. S. Pederson, *N. Y. Agr. Expt. Sta. Tech. Bull.*, **167**, 3 (1930).

(223) H. Fink and F. Just, *Biochem. Z.*, **296**, 307 (1938).

(224) A. Freund, *Monatsh.*, **2**, 636 (1881). See also A. A. Noyes and W. H. Watkins, *J. Am. Chem. Soc.*, **17**, 890 (1895).

(225) C. Neuberg and E. Färber, *Biochem. Z.*, **78**, 246 (1916); C. Neuberg and E. Reinfurth, *ibid.*, **92**, 254 (1918).

(226) E. Voisenet, *Ann. inst. Pasteur*, **28**, 807 (1914).

(227) C. H. Werkman and G. F. Gillen, *J. Bact.*, **23**, 167 (1932).

(228) K. Schutt, *Oesterr. Chem. Zt.*, **30**, 170 (1927).

(229) H. J. Deuel and Margaret G. Morehouse, *Advances in Carbohydrate Chem.*, **2**, 319 (1946).

of butyl bacteria pyruvic acid aldol may yield, besides butyric acid itself, higher homologs of butyric acid with even-numbered carbon chains, namely, caproic, caprylic and capric acids. Pyruvic acid and acetaldehyde could thus be assigned at an early period[230] their central position in the intermediary metabolism of plants and animals, as generally assumed today. These observations are the reason for placing them in the center of a scheme by Haehn and Kinttoff[231] in which the higher fatty acids are derived from these two sugar degradation products by means of repeated aldol condensations. Kluyver,[232] Reichel and Schmidt,[233] Smedley-Maclean,[234] Fink, Haehn and Hoerburger,[235] Geffers,[236] Damm,[237] Schwartz,[238] and others contributed special material to the question of synthesizing fat from sugar degradation products. Kleinzeller[239] states that acetaldehyde is used for fat synthesis by *Torulopsis lipofera.* By means of the captation method, acetaldehyde can be identified in microorganisms as an intermediate in fat synthesis. No D-glucose is used for this process under anaerobic conditions. The synthesis of fat is closely bound up with aerobic processes; phosphorylation takes a part, and iodoacetate inhibits the production of fat. It can be formed from D-glucose, D-fructose, maltose and ethanol. Smythe[240] has found that baker's yeast and the yeast-like *Endomycopsis* form fat from pyruvate and lactate, which are sugar degradation products. Recently it has been asserted[240a] that these condensations may have their origin also in acetic acid which is in turn derived from ethanol via acetaldehyde. On the whole, oxydation-reduction processes are involved and the synthetic reactions are exergonic.

(230) C. Neuberg, in "Handbuch der Biochemie," (C. Oppenheimer, Editor), Gustav Fischer, Jena, Ergänzungsband, p. 569 (1913); "Festschrift der Kaiser Wilhelm Gesellschaft," Verlag Julius Springer, Berlin, p. 170 (1921); *Ber.*, **55,** 3635 (1922).

(231) H. Haehn and W. Kinttof, *Ber.*, **56,** 439 (1923). *Chem. Zelle u. Gewebe*, **12,** 115 (1925). Theories about the origin of higher fatty acids from acetaldehyde, crotonaldehyde and α,β-hexylenaldehyde, but not supported by biochemical experiments, are due to M. von Nencki, *J. prakt. Chem.* [2] **17,** 105 (1878), *Opera omnia I,* **387** (1905), and to T. Curtius and H. Franzen, *Ann.*, **390,** 120 (1912).

(232) A. J. Kluyver, *Arch. Mikrobiol.*, **1,** 190 (1930).

(233) L. Reichel and O. Schmidt, *Biochem. Z.*, **300,** 274 (1939).

(234) Ida Smedley-Maclean and Dorothy Hoffert, *Biochem. J.*, **20,** 346 (1926); Ida Smedley-Maclean, *Ergeb. Enzymforsch.*, **5,** 285 (1936).

(235) H. Fink, H. Haehn and W. Hoerburger, *Chem. Ztg.*, **61,** 689, 723, 744 (1937).

(236) H. Geffers, *Arch. Mikrobiol.*, **8,** 66 (1937).

(237) H. Damm, *Chem. Ztg.*, **67,** 47 (1943).

(238) W. Schwartz, *Z. angew. Chem.*, **50,** 294 (1937).

(239) A. Kleinzeller, *Biochem. J.*, **38,** 480 (1944).

(240) C. V. Smythe, *J. Biol. Chem.*, **125,** 635 (1938).

(240*a*) B. T. Bornstein and H. A. Barker, *J. Biol. Chem.*, **172,** 659 (1948).

The term "fat coefficient," introduced by Rippel,[241] is a measure of the number of grams of fat formed per 100 grams of sugar consumed. The highest yield of fat from D-glucose that has been obtained experimentally, with a yeast from soil, was 15.6%. More recently it has been possible[242] to cultivate yeast cells from *Rhodatorula gracilis* which contained as much as 63.2% of fat in the dry substance. Four and one-half grams of D-glucose is required for the formation of 1 g. of fat in *Rhodatorula gracilis*, which means that 22% of the sugar is converted into fat. Damm[237] and also Mull and Nord,[197a] who used different methods and different microorganisms, have been able to produce large amounts of fat from pentoses and from hexoses and hydrolyzable disaccharides. Fat formation takes place very rapidly and up to 50% of the dry substance may consist of fat. It may be worth recalling that the formation of fat from the lowest sugar, glycolaldehyde, was observed at an early date.[234]

Lemoigne's[243] findings on lipids deserve attention because of their relationship to the true butylogenic fermentations as a phenomenon of a "*fermentation β-hydroxybutyrique.*" A series of microbes, for example, *B. megatherium*, *B. mycoides*, *B. cereus*, *B. anthracis* and *Azotobacter chroococcum*, accumulate lipids which have the formula $(C_4H_6O_2)_n$; they are levorotatory anhydrides of β-hydroxybutyric acid and are soluble in alcohol and chloroform. On saponification with alkali and also through autolysis of the bacterial mass, they are transformed into both β-hydroxybutyric acid and α-crotonic acid. These polymerized etholides are formed from carbohydrates and as intracellular lipids may account for 50% and more of the dry substance of the bacilli. Out of 100 moles of D-glucose, 42 can be utilized for the synthesis of the polymeric lactides, acetylmethylcarbinol being formed as a by-product.

It may be stated at this point that the presence of a β-hydroxybutyrate fat in certain organisms is a matter of general biochemical importance. Usually β-hydroxybutyric acid and the acetone bodies are derived from *n*-butyric acid directly. The unambiguous formation of β-hydroxybutyric acid anhydrides from carbohydrates opens up new vistas; its formation from acetaldehyde, and from pyruvic acid, through aldol intermediates can be understood without difficulty. Kirrmann's[244] reaction, to which little attention has been paid, is at the same time an example of an oxygen shift, leading from hydroxyaldehydes to fatty acids.

(241) A. Rippel, *Arch. Mikrobiol.*, **11,** 271 (1940); *Naturwissenschaften*, **31,** 248 (1943).

(242) L. Enebo, L. G. Anderson and H. Lundin, *Arch. Biochem.*, **11,** 383 (1946). See also R. L. Starkey, *J. Bact.*, **51,** 33 (1946).

(243) M. Lemoigne, *Ann. Inst. Pasteur*, **39,** 144 (1925); *Helv. Chim. Acta*, **29,** 1303 (1946).

(244) A. Kirrmann, *Compt. rend.*, **185,** 1482 (1927); **186,** 701 (1928).

A substance which proved to be a polymer of crotonic acid, also possessing the formula $(C_4H_6O_2)_n$ that has just been mentioned, has been extracted from certain purple bacteria.[245] It is formed during the metabolism of some photosynthesizing bacilli and occurs as a reduction product of compounds of carbohydrate nature.

The aldehyde of β-hydroxybutyric acid, acetaldol, has been reported as one of the products of metabolism of human tubercle bacilli by Kasuya;[246] it is present particularly in the acetone-soluble fat moiety.

Finally, reference may be made to instructive publications by van Niel[247] and Gaffron,[245] showing a relationship between photochemical and phytochemical reductions.

(245) H. Gaffron, *J. Gen. Physiol.*, **26**, 195 (1942), and in "Currents in Biochemical Research" (D. E. Green, editor), Interscience Publishers, New York, p. 25 (1946).

(246) J. Kasuya, *J. Biochem. Japan*, **27**, 284 (1938).

(247) C. B. van Niel, *Advances in Enzymol.*, **1**, 263 (1941). *Bact. Revs.*, **8**, 1 (1944).

THE ACYLATED NITRILES OF ALDONIC ACIDS AND THEIR DEGRADATION

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Contents

I. Introduction

On January 9, 1893 Alfred Wohl read a paper[1] before the Deutschen Chemischen Gesellschaft on the "Abbau des Traubenzuckers." In it he developed ideas formulated two years earlier on the possibility of degrading aldoses through their oximes.[2] He described the transformation of D-glucose into D-arabinose, the first example of a reaction, now known as the Wohl degradation, that allows the transformation of one aldose into another with one less carbon atom. Although the classical Wohl degradation is no longer employed for preparative purposes, the study of its interpretation and different variations has held the attention of the chemist up to the present time and is of permanent interest.

The first step in the degradation is the preparation, starting from an aldose, of the acylated nitrile of an aldonic acid. The nitrile and the acyl groups may be removed by various methods to yield a new aldose,

(1) A. Wohl, *Ber.*, **26**, 730 (1893).

(2) A. Wohl, *Ber.*, **24**, 994 (1891).

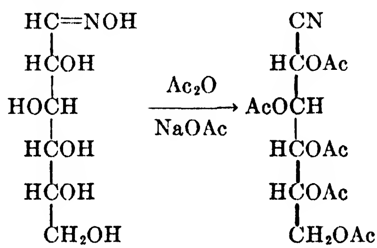
or its derivative, with one less carbon atom. A carbon atom of the original sugar is removed in the process and appears in the product as a salt of hydrocyanic acid. The different steps will be considered in detail.

II. The Nitriles of the Aldonic Acids

1. *The Acylated Nitriles*

Wohl prepared the acylated nitriles of the aldonic acids by heating the aldose oximes with a mixture of sodium acetate and acetic anhydride. With careful control of the reaction, this method may be used for preparative work with fairly good results.

In his first paper Wohl[1] reported the conversion of D-glucose oxime (I) into pentaacetyl-D-gluconontrile (II).



I II

Afterward, Behrend[3] found that the nitrile is also produced when D-glucose oxime is treated with pyridine and acetic anhydride, and this method has been extended to the preparation of other nitriles of aldonic acids.[4]

The formation of nitriles from aldoximes was at that time a well-known reaction, at least in its general lines. Gabriel and Meyer[5] found that *o*-nitrobenzonitrile was obtained by heating *o*-nitrobenzaldoxime, prepared in an indirect way, with sodium acetate and acetic anhydride; Lach[6] prepared benzonitrile in a similar way, and Dollfuss[7] transformed aldoximes of the aliphatic series into nitriles by simple treatment with acetic anhydride. The complicated character of this reaction was demonstrated by the classical work of Beckmann and Hantzsch,[8] who

(3) R. Behrend, *Ann.*, **353**, 106 (1907).

(4) J. R. Mendive, *Chemia*, **6**, 321 (1930); V. Deulofeu, P. Cattaneo and G. Mendivelzua, *J. Chem. Soc.*, 147 (1934); E. Restelli de Labriola and V. Deulofeu, *J. Am. Chem. Soc.*, **62**, 1611 (1940).

(5) S. Gabriel and R. Meyer, *Ber.*, **14**, 2332 (1881).

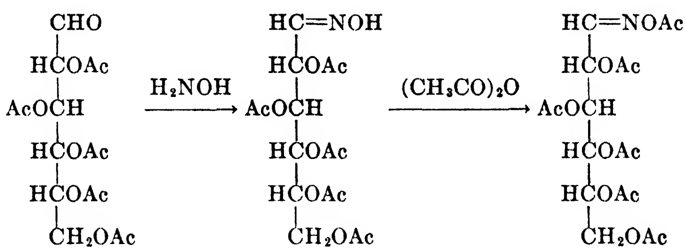
(6) B. Lach, *Ber.*, **17**, 1571 (1884).

(7) W. W. Dollfuss, *Ber.*, **25**, 1917 (1892).

(8) For further information on the oximes see: R. L. Shriner, R. Adams and C. S. Marvel in "Organic Chemistry" (H. Gilman, editor), John Wiley and Sons,

showed that aldoximes can exist in two structural forms. Subsequently Hantzsch[9] found that the acylated β-oximes, that we now know have the *anti* structure, are easily transformed into nitriles, whereas the acylated α-(*syn*) forms are not, nitriles being obtained only after transformation of *syn* into *anti* structures. This statement has been confirmed in a general way by many subsequent workers, and it is a general belief that *anti* oximes yield nitriles easier than the *syn* forms.

In the case of the oximes of the aldose sugars, the situation is more complicated because of the possibility of both open-chain and cyclic structures. That aldose oximes can react in the open-chain form follows from the formation of the nitriles and from the isolation of acylated open-chain aldose oximes as secondary products in preparation of nitriles. For example, Wolfrom and Thompson,[10] by the action of sodium acetate-acetic anhydride on D-glucose oxime, not only obtained pentaacetyl-D-glucon
onitrile, in 40% yield, but also isolated a small amount of hexaacetyl-*aldehydo*-D-glucose oxime (V) identical with that prepared by mild acetylation of pentaacetyl-*aldehydo*-D-glucose oxime (IV) whose structure was assured by its formation from pentaacetyl-*aldehydo*-D-glucose (III).



III IV V

This hexaacetyl-*aldehydo*-D-glucose oxime was easily transformed into the nitrile by heating at 135–140°, or by treatment with sodium acetate-acetic anhydride, and Wolfrom and Thompson considered the open-chain oxime and its acetylated derivative to be an intermediate in the production of the nitrile. We have no proof of the original structure of the oxime. Under the conditions of temperature employed, even a *syn* oxime can be transformed into the nitrile. If we accept the assumption that the isolated open-chain oxime is the only intermediate, its

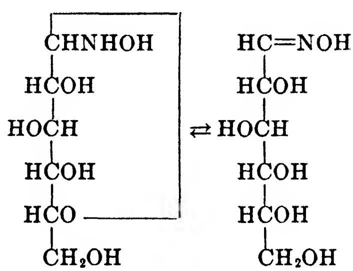
New York, 2nd. ed., Vol. II, p. 465 (1943); J. Meisenheimer and W. Theilacker in "Stereochemie" (K. Freudenberg, editor), F. Deuticke, Leipzig, p. 963 (1933).

(9) A. Hantzsch, *Ber.*, **24**, 35 (1891).

(10) M. L. Wolfrom and A. Thompson, *J. Am. Chem. Soc.*, **53**, 622 (1931).

stability points to a *syn* structure, but even in that case we lack a direct proof.

When mild conditions, such as pyridine-acetic anhydride in the cold, are employed for acetylation of the oxime, some monosaccharide oximes yield nitriles exclusively;[4] their formation can be interpreted as resulting from a very reactive oxime having an *anti* structure. As Hauser and Jordan[11] have shown, pyridine facilitates the transformation of acetylated *anti* oximes into nitriles. With other sugars this type of mild acylation gives large amounts of acylated *aldehydo* oximes that are stable and can be transformed into the nitrile only by a more drastic treatment. Although these stable oxime acetates seem to be *syn* forms, we do not have any direct proof of their structure. The *aldehydo* oxime structures (VII) are derived from the ring oximes (VI) through a prototropic change:



VI VII

Evidence that aldose oximes can have a ring structure under certain conditions, is based on their mutarotation in aqueous solution and also on chemical evidence. The mutarotation of aldose oximes was first observed by Jacobi[12] and subsequently extended by other workers.[13] Von Miller and Plöchl[14] found that D-glucose oxime, the most thoroughly investigated of the sugar oximes, did not add hydrogen cyanide as the true oximes do. Also, Irvine and Gilmour,[15] upon methylation, obtained a pentamethyl derivative, to which a cyclic structure, now written as VIII, was assigned, instead of the expected hexamethyl compound IX, derived from the open-

(11) G. R. Hauser and F. Jordan, *J. Am. Chem. Soc.*, **58,** 1772 (1936).

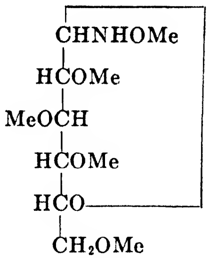
(12) H. Jacobi, *Ber.*, **24,** 696 (1891).

(13) D-Galactose oxime, M. L. Wolfrom, A. Thompson and L. W. Georges, *J. Am. Chem. Soc.*, **54,** 4091 (1932); D-gala-L-*manno*-heptose oxime, R. M. Hann and C. S. Hudson, *ibid.*, **59,** 1898 (1937); D-arabinose oxime, R. C. Hockett and C. W. Maynard, Jr., *ibid.*, **61,** 2111 (1939); D-altrose oxime, R. C. Hockett and L. B. Chandler, *ibid.*, **66,** 627 (1944).

(14) W. von Miller and J. Plöchl, *Ber.*, **27,** 1281 (1894).

(15) J. C. Irvine and R. Gilmour, *J. Chem. Soc.*, **93,** 1439 (1908); J. C. Irvine and Agnes M. Moodie, *ibid.*, **93,** 95 (1908).

chain form. By hydrolysis tetramethyl-D-glucose, now written as X, was produced.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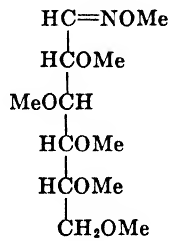
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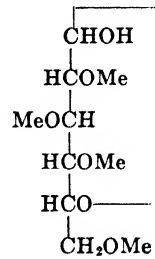
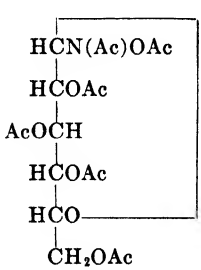

X

Acetylated aldose oximes with a ring structure have also been obtained during the preparation of the nitriles, both with sodium acetate-acetic anhydride and with pyridine-acetic anhydride. Thus it appears that under these conditions at least a certain amount of the oxime is present in the ring form.

Wohl[1] isolated a hexaacetyl-D-glucose oxime from the mother liquor obtained during the preparation of pentaacetyl-D-gluconназванonitrile. The same acetylated oxime was prepared by Behrend.[3] Wolfrom and Thompson[10] studied it further, and showed conclusively that it possessed a ring structure XI, for it could not be transformed into a nitrile. Because of its low specific rotation, it was assigned to the β-D series.

A cyclic hexaacetyl oxime (XII) was also isolated by mild acetylation of D-galactose oxime and by treatment with sodium acetate and acetic anhydride.[16] A cyclic aldoxime acetate (XIII) was also obtained by Votoček[17] from D-fucose oxime, and by Restelli de Labriola and Deulofeu[18]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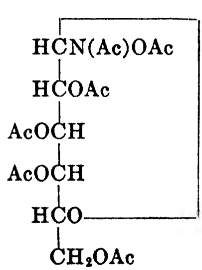
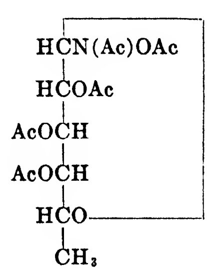

XII


XIII

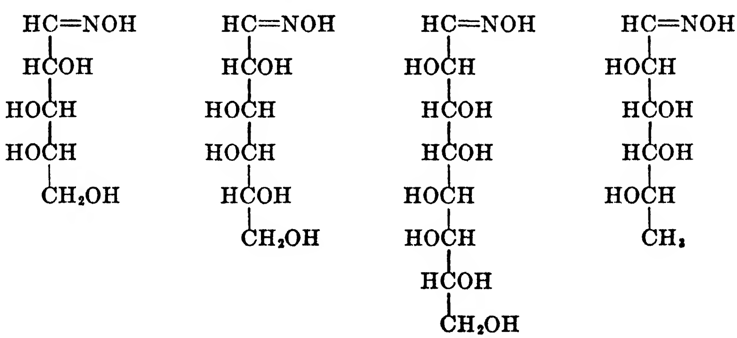
(16) V. Deulofeu, M. L. Wolfrom, P. Cattaneo, C. C. Christman and L. W. Georges, *J. Am. Chem. Soc.*, **55**, 3488 (1933).

(17) E. Votoček, *Ber.*, **50**, 35 (1917).

(18) E. Restelli de Labriola and V. Deulofeu, *J. Am. Chem. Soc.*, **62**, 1611 (1940).

from L-fucose oxime. A tentative correlation of configuration and reaction product for aldose oximes under mild conditions of acetylation, was first made by Hann and Hudson,[13] who pointed out that D-gala-D-*manno*-heptose oxime gives a heptaacetyl-*aldehydo*-D-gala-D-*manno*-heptose oxime and behaves like mannose oxime[4] which has the same spatial configuration about carbon atoms 2 to 5, inclusive. An extension of the correlation was made by Restelli de Labriola and Deulofeu[18] and additional information is now available. The pentose oximes can be made the starting point of such a correlation. Upon mild acetylation, L-arabinose oxime (XIV),[19] gives only the acetylated nitrile. This high reactivity was also found in tetraacetyl-*aldehydo*-L-arabinose oxime, which, under mild conditions of acetylation, yielded only the nitrile.[16]

The spatial configuration about carbon atoms 2, 3 and 4 of L-arabinose is duplicated in the oxime of D-galactose (XV),[16] which, on mild acetylation, gives principally the acetylated nitrile and the *aldehydo* oxime acetate. The enantiomorphous configuration of carbon atoms 2, 3 and 4 is present in D-gala-L-*gala*-octose oxime (XVI).[20] Acetylation of the oxime has not been attempted in the case of the octose, but when the heptaacetyl-*aldehydo*-D-gala-L-*gala*-octose oxime was submitted to mild acetylation only the expected octaacetyl compound was obtained, and no nitrile was produced, a result quite different from that obtained upon acetylation of tetraacetyl-L-arabinose oxime.



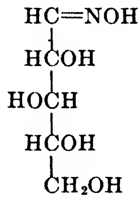
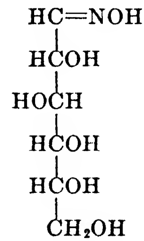
XIV XV XVI XVII

L-Fucose oxime (XVII), with the same spatial configuration as L-galactose oxime, is exceptional in that it gives a cyclic L-fucose oxime pentaacetate[18] at low temperature; the nitrile is formed when higher temperatures are employed.

(19) Formulas of the oximes are written in their *aldehydo* structures to facilitate comparisons among them.

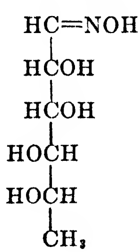
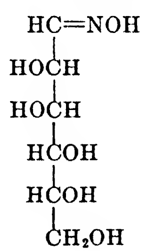
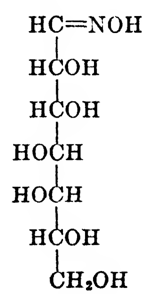
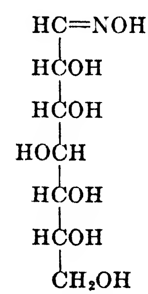
(20) R. M. Hann, W. D. Maclay and C. S. Hudson, *J. Am. Chem. Soc.*, **61**, 1270 (1939).

D-Xylose oxime (XVIII)[4] is also transformed into the nitrile even by mild acetylation. The configuration of carbon atoms 2, 3 and 4 is the same as in D-glucose oxime (XIX), but the latter reacts in a different form, giving almost exclusively the cyclic oxime upon mild acetylation. At higher temperatures the acetylated nitrile is produced.

XVIII XIX

The oxime of D-lyxose has not been prepared, but L-rhamnose oxime (XX) with the same spatial configuration about carbon atoms 2, 3 and 4 reacts in an open-chain form when submitted to mild acetylation,[4] and gives only the nitrile; this reactivity is confirmed when the oxime is submitted to mild propionylation, for only the hexapropionyl-*aldehydo*-L-rhamnose oxime is produced and that in high yield.[21] As already mentioned, the oximes of D-mannose (XXI),[4] and D-gala-L-*manno*-heptose (XXII),[13] having the same configuration as rhamnose about carbon atoms 2, 3 and 4, react in a similar manner.

XX XXI XXII XXIII

Another oxime with the same configuration about the three carbon atoms in question is D-gluco-D-*gulo*-heptose oxime (XXIII);[18] it also reacts in an open-chain form, but gives only the nitrile.

A special case is the exclusive formation of the nitrile by the mild acetylation of D-glucosamine oxime.[18]

Acetylated nitriles also have been prepared from free nitriles by

(21) F. Giménez, Thesis, Facultad de Ciencias Exactas, Físicas y Naturales, Buenos Aires, 1947.

acetylation. Mikšic[22] prepared the acetylated 7-desoxy-L-manno-L-*gala*-heptono- and D-manno-D-*gala*-heptononitriles and Wolfrom, Thompson and Hooper[23,23a] made the pentaacetyl derivatives of *N*-methyl-L-glucosaminonitrile and *N*-methyl-L-mannosaminonitrile, each by direct acetylation of the corresponding free nitrile.

The acetylated nitriles can also be obtained by dehydration of the corresponding amides. Phosphorus oxychloride is employed as the dehydrating agent. In this way Zemplén and Kiss[24] prepared hexa-acetyl-D-gluco-D-*gulo*-heptononitrile, and Ladenburg, Tishler, Wellman and Babson,[25] tetraacetyl-D-ribononitrile, tetraacetyl-D-arabononitrile and pentaacetyl-D-glucon準nitrile.

Propionylated nitriles have been obtained by Giménez[21] through the action of pyridine-propionic anhydride on aldose oximes. Although L-rhamnose oxime gave a crystalline pentapropionyl *aldehydo* oxime that could be transformed into the propionylated nitrile by heating, the oximes of D-glucose and D-galactose yielded only sirups, from which the corresponding propionylated nitriles could be isolated by heating and molecular distillation; these results showed that at least a part of the oxime reacts in the *aldehydo* form.

The first benzoylated nitrile of an aldonic acid was prepared by Brigl, Mühlschlegel and Schinle[26] by benzoylation of the D-manno-D-*gala*-heptononitrile; the hexabenzoyl derivative was obtained. A series of benzoylated nitriles has recently been prepared by Restelli de Labriola and Deulofeu[27] by treatment of the oximes of L-rhamnose, D-glucose, D-galactose and D-mannose with benzoyl chloride and pyridine. They were the sole products and were obtained in high yields.

2. *The Free Nitriles*

Free aldonic acid nitriles have been prepared in a few cases. Kiliani[28] obtained the cyanohydrin of D-fructose by adding hydrocyanic acid to the ketose and Mikšic[22] obtained the nitriles already mentioned in a similar way.

(22) J. Mikšic, *Vestnik Král. Čes. Spol. Nauk.*, Cl. II, 18 pp. (1926); *Chem. Abstracts*, **23**, 2941 (1929).

(23) M. L. Wolfrom, A. Thompson and I. R. Hooper, *J. Am. Chem. Soc.*, **68**, 2343 (1946).

(23*a*) M. L. Wolfrom and A. Thompson, *J. Am. Chem. Soc.*, **69**, 1847 (1947).

(24) G. Zemplén and D. Kiss, *Ber.*, **60**, 165 (1925).

(25) K. Ladenburg, M. Tishler, J. W. Wellman and R. D. Babson, *J. Am. Chem. Soc.*, **66**, 1217 (1944).

(26) P. Brigl, H. Mühlschlegel and R. Schinle, *Ber.*, **64**, 2921 (1931).

(27) E. Restelli de Labriola and V. Deulofeu, *J. Org. Chem.*, **12**, 726 (1947).

(28) H. Kiliani, *Ber.*, **18**, 3066 (1885); **19**, 221 (1886).

By hydrolysis of pentaacetyl-D-gluconontrile with sulfuric acid, Zemplén[29] obtained the free nitrile, and the same compound was prepared by Wohl and Wollenberg,[30] who treated D-glucose with hydroxylamine acetate and carried out the subsequent loss of acetic acid under rigidly controlled conditions. This last type of preparation can be correlated with the observation of Wolfrom and Thompson[10] that pentaacetyl-*aldehydo*-D-glucose oxime decomposes on heating without production of the nitrile, whereas the hexaacetate is easily transformed into the nitrile; this reaction emphasizes the importance of the acetylation of the oxime group for the formation of nitriles.

The free nitriles of *N*-methyl-L-glucosaminic acid and *N*-methyl-L-mannosaminic acid have been prepared by Wolfrom, Thompson and Hooper[23,23a] by the Kiliani-Fischer cyanohydrin synthesis.

In aqueous solution the free nitriles mutarotate in a complex manner[23,29] that indicates the existence of at least three compounds in the equilibrated solutions. This reaction has been interpreted as a reversible transformation of the nitriles into other substances.

3. *Properties of the Acylated Nitriles*

Acetylated nitriles of the aldonic acids are crystalline solids that are very soluble in most nonpolar organic solvents (chloroform, ether, benzene), less soluble in the alcohols usually employed for recrystallization, and almost insoluble in cold water; some compounds have a remarkably high solubility in water at elevated temperatures.

A rule stating that nitriles with an acetoxy group on the right of carbon atom 2 are dextrorotatory was formulated by Deulofeu,[31] and although it applies to most acetylated nitriles, some exceptions have been found. Propionylated nitriles are liquids or solids that can be molecularly distilled without decomposition.[21] Benzoylated nitriles are all solids.

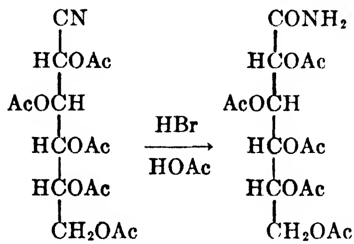
In addition to the chemical reactions employed in connection with the degradation reaction, which will be discussed later in detail, it should be mentioned that the nitriles can be transformed into amides (XXV) by treatment with hydrobromic acid in glacial acetic acid, a reaction first applied by Zemplén and Kiss[24] to pentaacetyl-D-gluconontrile (XXIV), and also used by subsequent workers.[32]

(29) G. Zemplén, *Ber.*, **60**, 171 (1927); P. E. Papadakis and H. J. Cohen, *J. Am. Chem. Soc.*, **60**, 765 (1939); P. E. Papadakis, *ibid.*, **64**, 1950 (1942).

(30) A. Wohl and O. Wollenberg, *Ann.*, **500**, 281 (1932).

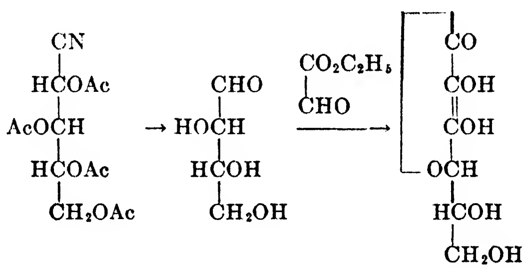
(31) V. Deulofeu, *Nature*, **131**, 548 (1933).

(32) C. D. Hurd and J. C. Sowden, *J. Am. Chem. Soc.*, **60**, 235 (1938); G. Zemplén, E. Balarsa and M. Gardonyi, *Ber.*, **71**, 768 (1938); V. Deulofeu and E. Restelli de Labriola, *J. Am. Chem. Soc.*, **61**, 1110 (1939).



XXIV XXV

The nitriles can also be employed as intermediates in the condensation of ethyl glyoxylate with *aldehydo* sugars under the influence of sodium methoxide, leading to the preparation of L-ascorbic acid and similar compounds. Helferich and Peters[33] described the synthesis of D-ascorbic acid (XXVIII) from tetraacetyl-D-xylononitrile (XXVI); presumably the nitrile underwent a type of Zemplén degradation and the D-threose (XXVII) thus formed condensed with the glyoxylic ester. The method



XXVI XXVII XXVIII

has been patented[34] and in a later patent[35] it is stated that ethyl glyoxylate can be substituted by mesoxalic esters.

4. *Experimental Procedures*

Preparation of nitrile acetates from oximes with sodium acetate and acetic anhydride. Pentaacetyl-D-gluconoitrile.[24] If only the nitrile is needed, isolation of the oxime can be avoided. One hundred grams of anhydrous D-glucose was dissolved in 50 ml. of warm water, and maintaining the temperature at 60°, a solution of 28 g. of hydroxylamine in 700 ml. of ethanol was added sufficiently slowly that no precipitation took place. After one hour at 65°, the reaction mixture was concentrated under reduced pressure to a thick sirup. The residue was mixed with absolute ethanol, the ethanol evaporated and the operation repeated in order to eliminate all water. One hundred and twenty grams of anhydrous sodium acetate and 700 ml. of acetic anhydride were added to the sirup, and the mixture was slowly and cautiously warmed in a water bath to 95°. It was advisable to agitate the flask continuously and to watch the

(33) B. Helferich and O. Peters, *Ber.*, **70**, 465 (1937).
(34) B. Helferich, German Pat. 637,448 (1936); *Chem. Abstracts*, **31**, 709 (1937).
(35) B. Helferich, U. S. Pat. 2,707,680 (1940); *Chem. Abstracts*, **34**, 8184 (1940).

temperature closely. If a rapid rise in temperature occurred, the flask was immersed in ice water; when the temperature had dropped, heating was resumed. When the reaction had apparently ceased and the sirup had dissolved, the solution was kept at 90° for one hour and then poured into three liters of ice water. The suspension was well agitated and the oily precipitate usually solidified on standing overnight. The crystals were separated, washed with water and recrystallized from 300 ml. of ethanol; yield, 115 g., m. p. 84°. From the mother liquors an additional 5 g. of impure nitrile pentaacetate could be obtained; total yield, 56.8%.

*Preparation of acetylated nitriles from amides. Tetraacetyl-*D*-ribononitrile.*[25] A mixture of 5 g. of tetraacetyl-D-ribonoamide, 6 g. of phosphorus oxychloride and 20 ml. of alcohol-free chloroform was refluxed for three hours. The cooled solution was cautiously stirred into 50 ml. of ice water, and the chloroform layer separated. The aqueous fraction was extracted with three 20-ml. portions of chloroform, and the combined chloroform extracts were washed with ice water and aqueous sodium bicarbonate solution. After drying over anhydrous sodium sulfate, the chloroform was completely removed by distillation under reduced pressure and the crystalline residue was recrystallized from 10 ml. of ether; yield 4.4 g. (92%), m. p. 66–68°. A second crystallization from ether or methanol gave a product which melted at 71–72°.

*Preparation of acetylated nitriles from oximes with pyridine-acetic anhydride. Pentaacetyl-*D*-glucosaminonitrile.*[18] One gram of D-glucosamine oxime hydrochloride was treated with 6 ml. of pyridine and 6 ml. of acetic anhydride and kept at 20° until solution had occurred. The solution was then concentrated and the crystalline residue was recrystallized from ethanol; yield 1.8 g. (84%), m. p. 126°.

*Preparation of propionylated nitriles from oximes. Pentapropionyl-*D*-glucononitrile.*[21] Twenty grams of D-glucose oxime was dissolved in 100 ml. of pyridine and 100 ml. of propionic anhydride was added; the solution was heated for one hour at 100° and was then poured into ice water. The sirupy precipitate was extracted with ether, and the ether solution was washed with sodium bicarbonate solution (5%) and with water. The ether was evaporated and the remaining sirup was distilled at 0.001 mm. at a bath temperature of 140°. The distillate solidified in crystalline form; yield 14.1 g. (59.8%), m. p. 58–60°. After purification by recrystallization from ethanol, the product weighed 9.7 g. and melted at 67–69°. After further recrystallization, a product of melting point 68–70° was obtained.

*Preparation of the benzoylated nitriles from oximes. Pentabenzoyl-*D*-galactononitrile.*[27] Five grams of finely divided D-galactose oxime was suspended in 30 ml. of pyridine, and 28 ml. of benzoyl chloride was slowly added. Heat developed and the oxime gradually dissolved. After twenty-four hours the partly solidified mass was poured into water; the precipitated sirup solidified after repeated washing with cold water. The solid was then filtered, dried well and recrystallized from acetic acid; yield, 16.2 g. (98%), m. p. 142–144°. After repeated recrystallization from ethanol, long needles melting at 144° were obtained.

III. Degradation of the Nitriles of the Aldonic Acids

1. *The Wohl Degradation*

The work which Wohl reported in his earliest paper on sugar degradation[2] had been planned with the expectation that the nitriles of the aldonic acids would react as cyanohydrins. When he carried out his first degradation with pentaacetyl-D-glucononitrile (XXIX)[1] he used ammonia

containing dissolved silver oxide in order to produce a simultaneous separation of the nitrile and a saponification of the acetyl groups, with the hope of obtaining a free pentose. Instead, a nitrogen-containing compound was isolated to which formula XXX was correctly assigned and the substance was named D-arabinose diacetamide. The degradation was the reversal of the classical Kiliani-Fischer synthesis.[36]

$$
\begin{array}{ccc}
\mathrm{CN} & & \\
| & & \\
\mathrm{HCOAc} & & \mathrm{HC(NHAc)_2} \\
| & & | \\
\mathrm{AcOCH} & \xrightarrow[\mathrm{Ag_2O}]{\mathrm{NH_3}} & \mathrm{HOCH} \\
| & & | \\
\mathrm{HCOAc} & & \mathrm{HCOH} \\
| & & | \\
\mathrm{HCOAc} & & \mathrm{HCOH} \\
| & & | \\
\mathrm{CH_2OAc} & & \mathrm{CH_2OH} \\
\mathrm{XXIX} & & \mathrm{XXX}
\end{array}
$$

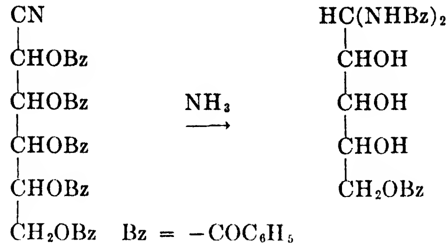
Ammonia with dissolved silver oxide was employed as the only degradation reagent until Maquenne[37] showed that ammonia alone could be employed with the same success; this method has been developed further by Hockett.[38]

Propionylated aldononitriles, prepared by Giménez,[21] have been degraded in a similar way and the dipropionamide derivatives of the lower sugars obtained without difficulty.

Brigl, Mühlschlegel and Schinle[26] degraded hexabenzoyl-D-manno-D-*gala*-heptononitrile, and this compound remained for many years the only benzoylated nitrile that underwent the reaction; D-mannose dibenzamide was obtained together with a D-mannose monobenzamide whose formation the authors attributed to a side reaction; this supposition merits reinvestigation in view of the findings of Hockett and Chandler[13] with hexaacetyl-D-gluco-D-*gulo*-heptononitrile.

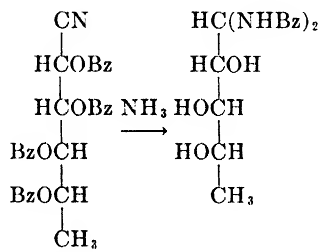
Restelli de Labriola and Deulofeu[27] have degraded benzoylated nitriles of the hexose series and tetrabenzoyl-L-rhamnononitrile, with ethanolic ammonia. The dibenzamide compounds of the lower sugars were obtained, but in the case of compounds with a benzoylated primary hydroxyl group, such as pentabenzoyl-D-glucono-, D-mannono- and D-galactononitriles (XXXI) the benzoyl group that esterifies the terminal hydroxyl was not removed (XXXII).

(36) C. S. Hudson, *Advances in Carbohydrate Chem.*, **1,** 2 (1945).
(37) L. Maquenne, *Compt. rend.*, **130,** 1402 (1900); *Ann. chim.*, [7] **24,** 399 (1901).
(38) R. C. Hockett, *J. Am. Chem. Soc.*, **57,** 2265 (1935).



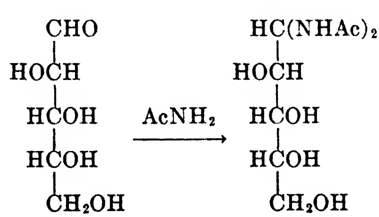
XXXI XXXII

With tetrabenzoyl-L-rhamnononitrile (XXXIII), a normal 5-desoxy-L-arabinose dibenzamide (XXXIV) was obtained.



XXXIII XXXIV

The Wohl degradation can be considered to consist of two steps: (a) the elimination of the acetyl and nitrile groups by the action of ammonia or ammoniacal silver oxide, and (b) the formation of the diacetamide compound. The formation of the latter was explained by Wohl on the basis of the intermediate formation of *aldehydo*-D-arabinose (XXXV), whose free aldehyde group condensed with acetamide, formed during the first step of the reaction, and gave D-arabinose diacetamide (XXXVI).

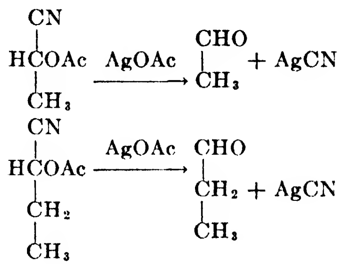


XXXV XXXVI

When Wohl published his first paper it was known that the action of ammonia on esters resulted in the liberation of the acyl groups as amides, and simple acetylated hydroxynitriles had been prepared.[39]

(39) L. Henry, *Compt. rend.*, **102**, 768 (1886).

However, experiments which may be considered as simplified models of the degradation were not carried out until some years later. In 1897, Colson,[40] working with the acetates of lactic nitrile and α-hydroxybutyric nitrile, showed that treatment with silver acetate removed the cyano groups and that acetic acid and acetaldehyde or propionaldehyde were produced.

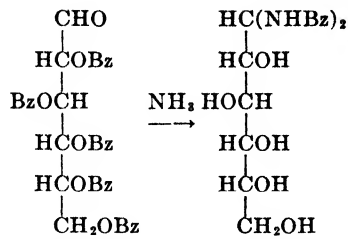


Although these experiments emphasized the pseudohalogen nature of the nitrile group when united to a carbon atom carrying a potential hydroxyl group, they are not exactly related to the Wohl degradation, especially since ammonia was not employed; apparently it was this reagent that led, in the final instance, to the production of the diacetamide compounds. Fenton[41] sometime later degraded acetylglycolonitrile, $AcOCH_2CN$, with ammonia and silver oxide, and, although silver cyanide was isolated, formaldehyde was only obtained after hydrolysis of the other reaction products, and there was some doubt as to whether methylene diacetamide or hexamethylenetetramine was formed.

Examples of the condensation of amides with aldehydes were also known at this time. Roth[42] and Schuster,[43] working in Strecker's laboratory, had prepared benzylidene diacetamide and anisylidene diacetamide by heating the aldehydes with acetamide. Von Richter[44] in 1872 reported that Tawildarow obtained ethylidene diacetamide by heating acetaldehyde and acetamide, and Nencki[45] obtained ethylidene dibenzamide by carrying out a similar reaction in the presence of hydrochloric acid.

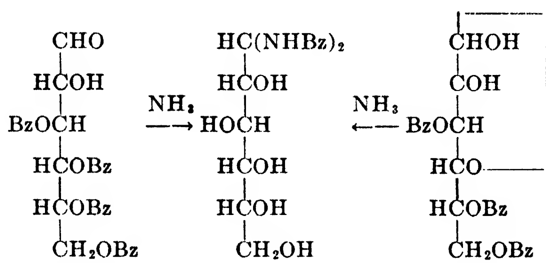
Evidence that the aldose diacetamides are derived from the union of preformed amides with the *aldehydo* form of the aldose was obtained many years later. Brigl, Mühlschlegel and Schinle,[26] obtained D-glucose dibenzamide (XXXVIII) by the action of methanolic ammonia on pentabenzoyl-*aldehydo*-D-glucose (XXXVII). They studied the same reaction

(40) A. Colson, *Ann. chim.*, [7] **12**, 231 (1897).
(41) H. J. H. Fenton, *J. Chem. Soc.*, **77**, 1294 (1900).
(42) E. Roth, *Ann.*, **154**, 72 (1869).
(43) A. Schuster, *Ann.*, **154**, 80 (1869).
(44) V. von Richter, *Ber.*, **5**, 477 (1872).
(45) M. Nencki, *Ber.*, **7**, 158 (1874).



XXXVII XXXVIII

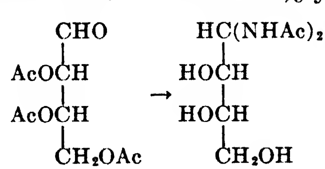
with the 3,4,5,6-tetrabenzoyl-*aldehydo*-D-glucose (XXXIX), a compound giving some of the reactions of a free aldehyde, and found that D-glucose dibenzamide was again produced; a similar reaction took place with 3,5,6-tribenzoyl-D-glucose (XL), to which a furanose structure was assigned. It was assumed that this compound could easily be transformed into the *aldehydo* form.



XXXIX XL

In the case of the above mentioned benzoyl-D-glucose derivatives a correlation was found between the formation of D-glucose dibenzamide and the permanent or transitory existence of a free aldehyde group. The same behavior was noted for triacetyl-*aldehydo*-L-erythrose (XLI), which by the action of ammonia produces L-erythrose diacetamide (XLII).[46]

Isbell and Frush[46a] have obtained a similar result in the case of tetraacetyl-*aldehydo*-L-arabinose which, when treated with methanolic ammonia, gave L-arabinose diacetamide in 53% yield.



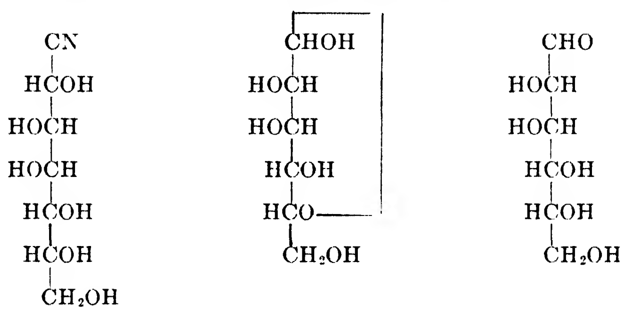
XLI XLII

(46) V. Deulofeu, *J. Chem. Soc.*, 2973 (1932).

(46*a*) H. S. Isbell and Harriet L. Frush, paper presented before the Division of Organic Chemistry at the Washington meeting of the American Chemical Society Aug. 30–Sept. 3, 1948. See also page 148.

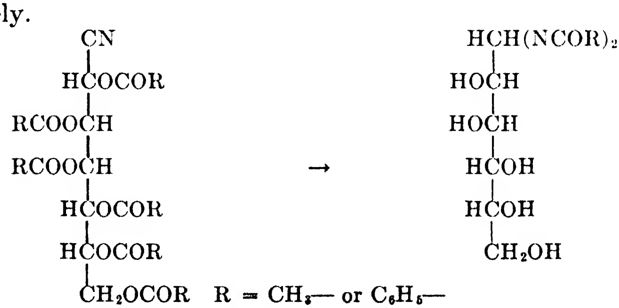
Negative evidence for the importance of the free aldehyde group is found in the failure of all efforts[26,47] to condense amides with free monosaccharides, which have a hemiacetal structure.

That the existence of a free aldehyde group is not the only condition for the production of these diamides follows from the work of Brigl, Mühlschlegel and Schinle[26] and of Hockett and Chandler.[47] Brigl, Mühlschlegel and Schinle[26] reported a case which shows the importance of the previous acylation of the nitrile, for determining the course of the degradation. They treated free D-manno-D-*gala*-heptononitrile (XLIII) with ammonia, silver nitrate and benzamide. D-Mannose (XLIV) was formed and isolated as the phenylhydrazone, but even if the *aldehydo*-D-



XLIII XLIV XLV

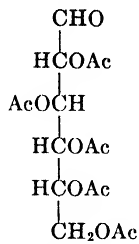
mannose (XLV) was formed no condensation took place with the benzamide present. In contrast, hexaacetyl-D-manno-D-*gala*-heptononitrile (XLVI) and the corresponding hexabenzoyl derivative were degraded to D-mannose diacetamide (XLVII) and D-mannose dibenzamide, respectively.

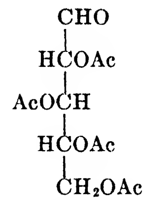


XLVI XLVII

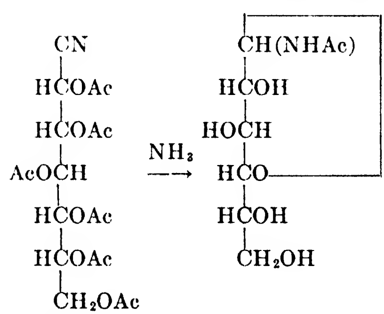
Hockett and Chandler[47] were unable to combine pentaacetyl-*aldehydo*-D-glucose (XLVIII) with acetamide or to obtain D-xylose diacetamide by the action of ammonia on tetraacetyl-*aldehydo*-D-xylose (XLVIIIa), in spite of the existence of a free aldehyde group in both substances.

(47) R. C. Hockett and L. R. Chandler, *J. Am. Chem. Soc.*, **66**, 957 (1944).


XLVIII


XLVIIIa

The diacetamide compounds were regularly obtained in all degradations employing ammonia with or without silver oxide until Hockett and Chandler[47] applied the method to hexaacetyl-D-gluco-D-*gulo*-heptononitrile (XLIX) and obtained a monoacetamide derivative that was identified as *N*-acetyl-D-glucofuranosylamine (L). The furanose structure of L was established by lead tetraacetate oxidation.[48] They


XLIX L

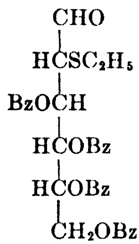
obtained the same compound by the direct action of ammonia on pentaacetyl-*aldehydo*-D-glucose, and Niemann and Hays[49] produced it by treating pentaacetyl-β-D-glucose with methanolic ammonia. As pentabenzoyl-*aldehydo*-D-glucose gave the dibenzamide compound, the importance of the acyl residue becomes evident.

A somewhat similar type of reaction was reported by Brigl, Mühlschleger and Schinle[26] who degraded hexabenzoyl-D-manno-D-*gala*-heptononitrile and obtained not only D-mannose dibenzamide, but also a D-mannose monobenzamide. The monobenzamide was thought to be a secondary product, arising from the action of nitric acid (produced while processing the reaction products) on the dibenzamide already present. This conclusion needs confirmatory evidence in view of the results of Hockett and Chandler.[47]

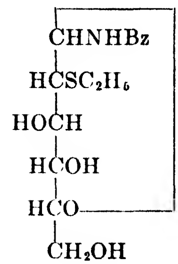
(48) R. C. Hockett, M. H. Nickerson and W. H. Reeder, *J. Am. Chem. Soc.*, **66**, 472 (1944).

(49) C. Niemann and J. T. Hays, *J. Am. Chem. Soc.*, **67**, 1302 (1945).

Brigl, Mühlschleger and Schinle[26] also obtained a monobenzamide by the action of ammonia on 2-thioethyl-3,4,5,6-tetrabenzoyl-*aldehydo*-D-glucose (LI). Without further evidence of its ring structure, they assumed this compound to be *N*-benzoyl-(2-thioethyl-D-glucopyranosyl)-



LI



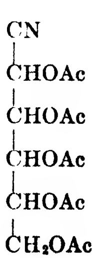
LII

amine (LII). In an analogous reaction, the formation of *N*-acetyl-D-glucofuranosylamine was reported by Hockett and Chandler.[47]

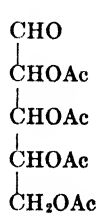
From these experiments it would appear that the presence of a free aldehyde group is a necessary but not a sufficient condition for the formation of diamides in the above reactions. Once formed, the aldehyde group must be stabilized long enough to allow it to react with one or more amide molecules, the latter arising from the ammonolysis of the ester groups present in the acylated sugar.

In view of the stability of the cyclic (hemiacetal) form of the aldoses, it is reasonable to assume that the open-chain *aldehydo* structure, formed by elimination of the nitrile group, will be more stable if cyclization is inhibited (by acyloxy groups on carbon atoms 4 or 5) than if cyclization may readily occur (hydroxyl groups on carbon atoms 4 or 5).

According to Hockett and Chandler,[47] the hydrolysis of an acetylated nitrile (LIII) results in the formation of an acetylated *aldehydo* sugar (LIV). If this compound then undergoes ammonolysis at carbon atoms 2 or 3, the formation of diacetamides is to be expected, since hemiacetal formation involving the C2 or C3 hydroxyl group is unknown. The

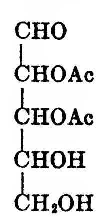


LIII



LIV

LV



LVI

diacetamide would then be formed by the condensation of acetamide with the free aldehyde group, followed by the elimination of the C4 and C5 acetyl groups.

On the other hand, if ammonolysis first occurred at carbon atoms 4 or 5, the formation of a cyclic hemiacetal would be favored, and the possibility of obtaining a diacetamide would be correspondingly decreased. Such an explanation accounts for the formation of *N*-acetyl-D-glucofuranosylamine reported by Hockett and Chandler.[47] Here, the first product of the reaction is a D-glucofuranose, which then condenses with acetamide. A similar explanation accounts for the results obtained by Brigl, Mühlschleger and Schinle[26] with 2-thioethyl-3,4,5,6-tetrabenzoyl-*aldehydo*-D-glucose.

An alternative explanation for the formation of monoacetamides has also been suggested by Hockett and Chandler, who point out that the monoamide could have been formed from the diamide by the loss of acetamide. However, this explanation seems unlikely in view of the fact that Niemann and Hays[49] have reported the preparation of *N*-acetyl-D-glucofuranosylamine by the action of ammonia on pentaacetyl-β-D-glucose, a reaction which involves the conversion of a pyranose to a furanose ring.

When a Wohl degradation is carried out in the laboratory, hydrolysis of the acyl and nitrile groups begins immediately upon addition of ammonia. The rate of hydrolysis of each of the groups present in the molecule is probably different and at the present time we have no knowledge of these rates of hydrolysis. As Hockett and Chandler have pointed out, the diacetamides are "obviously formed as a result of a delicate balance among the rates of several concurrent reactions."

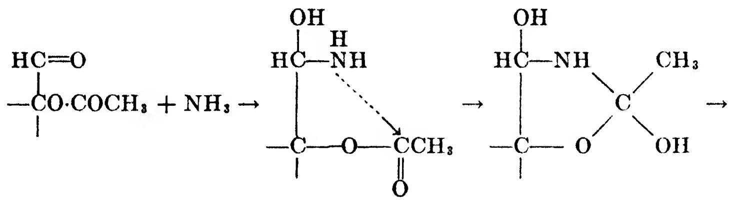
An indication of the sensitivity of the reaction to changes in the structure of the acylated nitrile is furnished by the work of Neuberg and Wolff,[50] who obtained hydrocyanic acid from the Wohl degradation of pentaacetyl-D-glucosaminonitrile, but could not obtain a diacetamide compound.

Recently Isbell and Frush[46a] have given an interpretation of the reactions leading to the formation of the diacetamide compounds. They admit that an aldehyde group is necessary.

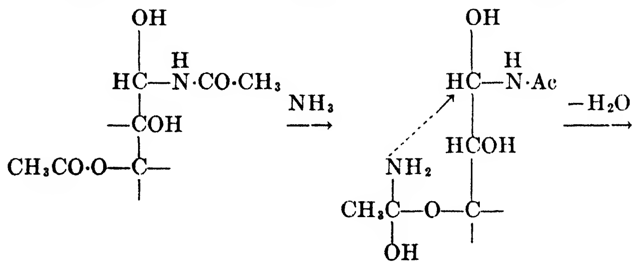
Ammonia then adds to the aldehydic carbon atom (LVIa) with formation of compound LVIb. The nitrogen of the amino group in a further phase of the reaction approaches the carboxylate carbon of the neighboring acetyl group and combines, forming a labile orthoester (LVIc). One of the possible forms of rearrangement of this orthoester is the migration of the acetyl to the nitrogen atom (LVId). The forma-

(50) C. Neuberg and H. Wolff, *Ber.*, **35**, 4009 (1902).

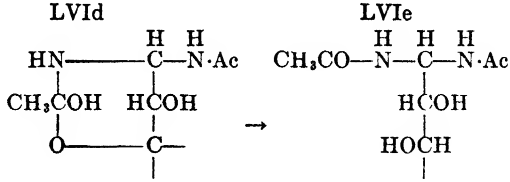
tion of the second acetamide group is explained by addition of ammonia to the carboxylate carbon of another *O*-acetyl group (LVIe) and rearrangement (LVIf and LVIg).



LVIa LVIb LVI c



LVId LVIe



LVIf LVIg

The formation of the diacetamide derivatives requires, in this theory, the existence of two acetyl groups so located in the molecule, that they can react, through the ammonia, with the aldehyde carbon.

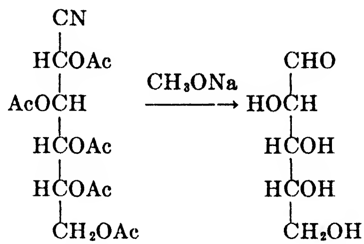
Although the acetyls on carbons 2 and 3 are depicted as forming the acetamide that remains combined to the aldehyde group, Isbell and Frush presume that any neighboring group could act in a similar way and that the selection of the groups involved in the reaction depends on the configuration of the sugar.'

2. *The Zemplén Degradation*

In 1926, Zemplén,[51] working with cellobiose, introduced a new type of degradation which, like the Wohl degradation, begins with the acetylated nitriles of the aldonic acids, but differs from the latter in the reagents used to bring about the removal of the nitrile and acetyl groups. Zem-

(51) G. Zemplén, *Ber.*, **59**, 1254 (1926).

plén employed sodium methoxide, which saponified the acetyl groups and removed the nitrile group as sodium cyanide. In the simpler case of pentaacetyl-D-glucononitrile (LVII),[24] the reaction may be summarized as follows:



LVII LVIII

The new aldose LVIII was obtained in solution, along with sodium acetate and by-products, and was isolated by means of an insoluble derivative. The method was developed especially for the degradation of disaccharides. Zemplén stated that the tentative application of a modified Wohl degradation led to unidentifiable products. The classical ammonia method was not applicable because it was impossible to hydrolyze the expected diacetamide compounds without at the same time hydrolyzing the new disaccharide. The use of sodium methoxide avoided this hydrolysis. Successive degradations allowed Zemplén to obtain evidence regarding the structure of some of the reducing disaccharides, a possibility to which Wohl[1] previously had called attention.

Recently, the use of two successive degradations by Zemplén's method has been applied to 3-(D-glucopyranosyl)-D-glucopyranose[51a] for the preparation of a non-reducing disaccharide, 1-(D-glucopyranosyl)-D-erythrofuranoside.

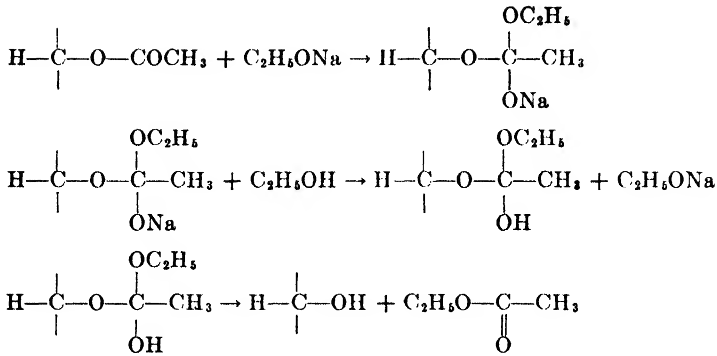
It appears that Fischer and Bergmann,[52] were the first workers to apply the well-known catalytic effect of the sodium alkoxides to the hydrolysis of acylated sugars in alcoholic solution.

A detailed study of some of the steps of the reaction was made by Zemplén and Kunz,[53] who proposed a mechanism to explain the very small amount of sodium ethoxide required for the saponification of the acetyl groups. They suggested that sodium ethoxide added to the carbonyl oxygen of the acetyl group, and was again regenerated with separation of the acetyl group as ethyl acetate, as in the following series of reactions.

(51*a*) A. M. Gakhokidze, *J. Gen. Chem.* (*U.S.S.R.*), **16,** 1923 (1946); *Chem. Abstracts*, **41,** 6210 (1947).

(52) E. Fischer and M. Bergmann, *Ber.*, **52,** 829 (1919).

(53) G. Zemplén and A. Kunz, *Ber.*, **56,** 1705 (1923).



(a)

(b)

(c)

In his later papers on degradation reactions, Zemplén employed sodium methoxide and used chloroform as the solvent for the sugar derivative. When the acyl groups are removed, the cyanohydrin can be considered an intermediate product which loses hydrocyanic acid to yield an aldose. It is possible that the acetyl and nitrile groups are eliminated simultaneously by concurrent reactions.

The nitrile group is very sensitive to alkalis and the elimination of it by the action of potassium hydroxide on acetaldehyde cyanohydrin was described by Simpson and Gauthier[54] as one of the reactions of the acetaldehyde cyanohydrin, a substance that they prepared for the first time. That the nitriles of the aldonic acids yield cyanides under the action of alkali, is described by Wohl[1] as one of the properties of pentaacetyl-D-gluconpremiumnitrile.

The method proposed by Zemplén has given in some cases, as in the degradation of D-glucose to D-arabinose, higher yields than Wohl's method, but the difficulty of isolation of the new sugars has hindered its application to preparative work. It has been extended to the propionylated nitriles by Giménez[21] and to benzoylated ones by Restelli de Labriola and Deulofeu.[27] Usually yields lower than with the acetyl derivatives have been obtained.

3. *Other Types of Degradation*

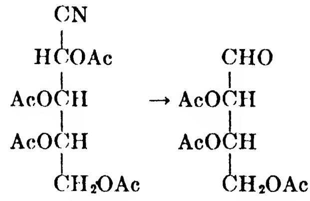
Apart from the general procedures that have been described, nitriles of aldonic acids are capable of other reactions that also lead to degradations.

An elimination of the nitrile and the acetyl group united to carbon 2, leaving intact the other acetyls, was achieved by Wohl[55] by treatment of tetraacetyl-L-arabononitrile (LIX) with silver oxide and a very small

(54) M. Simpson and A. Gauthier, *Compt. rend.*, **65**, 414 (1867).

(55) A. Wohl, *Ber.*, **32**, 3666 (1899).

amount of ammonia. Triacetyl-L-erythrose (LX) was produced in small yield.



LIX LX

Removal of the acetyl and nitrile groups by acid hydrolysis was also achieved by Wohl,[1] who isolated a pentosazone from the products from heating pentaacetyl-D-gluconositrile with 2 *N* hydrochloric acid. Fischer[56] also isolated what is now known to have been a 5-desoxy-L-arabinosazone from the products obtained by treatment of tetraacetyl-L-rhamnononitrile with 5% hydrochloric acid. At the same time partial transformation of the nitrile into the aldonic acid takes place as shown by Maquenne,[37] who obtained D-xylonic acid by treating tetraacetyl-D-xylononitrile with concentrated hydrochloric acid.

Zemplén and Kiss,[24] in the case of pentaacetyl-D-gluconositrile, removed the acetyl groups by heating the compound with sulfuric acid in ethanol, a procedure that left the nitrile almost intact. This group was subsequently eliminated with silver carbonate and D-arabinose was isolated as the diphenylhydrazone. This procedure was applied to other nitriles by Deulofeu and coworkers.[57,58]

The free D-gluconositrile was also degraded to D-arabinose by Wohl and Wollenberg[30] by simple heating of its solution and elimination of hydrocyanic acid with carbon dioxide gas.

4. *Experimental Procedures*

D-*Lyxose diacetamide.* (*Ammonia-silver oxide.*)[59] Ten grams of pentaacetyl-D-galactononitrile was dissolved in 30 ml. of ethanol, and a solution of silver oxide (from 5 g. of silver nitrate) in 50 ml. of 30% ammonia added. After two days at room temperature, the precipitated silver cyanide was removed by filtration and the solution evaporated *in vacuo* at 40° until all ammonia was eliminated. The residue was diluted with water and the soluble silver eliminated by treatment with hydrogen sulfide and filtration. The filtrate was treated with decolorizing carbon, filtered and evaporated to dryness. When the residue crystallized, it was suspended in warm ethanol and filtered; yield, 2.5 g. (40%). After recrystallization from 60% ethanol, the product had a melting point of 230–231°.

(56) E. Fischer, *Ber.*, **29**, 1377 (1896).

(57) V. Deulofeu and R. J. Selva, *J. Chem. Soc.*, 225 (1929); *Anales asoc. quím. argentina*, **17**, 13 (1929).

(58) V. Deulofeu, *J. Chem. Soc.*, 2602 (1930).

(59) A. Wohl and E. List, *Ber.*, **30**, 3101 (1897).

D-*Threose diacetamide.* (*Aqua ammonia.*)[58] Thirty grams of tetraacetyl-D-xylononitrile was mixed with 300 ml. of concentrated aqua ammonia (28–29%) and warmed on a water bath until the solid was all dissolved, and then allowed to stand for three hours. The solution was concentrated *in vacuo* to a thick sirup, which was dissolved in aqua ammonia and reconcentrated. The final thick sirup was dissolved in absolute ethanol and ether added to the point of turbidity. After forty-eight hours in a refrigerator the crystals which had formed were collected; yield, 16.5 g. (78%). They were recrystallized by dissolution in two volumes of warm 75% ethanol, filtration through carbon and the addition of an equal volume of absolute ethanol. The compound crystallized slowly, forming clear, sharp needles or prisms in rosets, m. p. 165–167°.

5-*Benzoyl*-D-*lyxose dibenzamide.* (*Ethanolic ammonia.*)[27] Three grams of finely powdered pentabenzoyl-D-galactononitrile was suspended in 90 ml. of 96% ethanol saturated with ammonia and shaken at room temperature. After two hours the nitrile had dissolved and shaking was continued for one hour. The solution was concentrated to dryness *in vacuo* and the dried residue was washed with cold ethanol until no more color was removed. The 5-benzoyl-D-lyxose diacetamide remaining melted at 210–218°. It could be purified by recrystallization from a large volume of ethanol and appeared as long needles melting at 222–224°.

Degradation of pentapropionyl-D-*gluconontrile.* (*Sodium methoxide.*)[21] Five grams of pentapropionyl-D-gluconontrile was dissolved in 7 ml. of chloroform cooled to −5° and then a cool solution of 0.84 g. of sodium in 10 ml. of methanol was added. A jelly-like mass was produced that was kept in the ice-salt bath for five minutes. Fourteen milliliters of water and 2.5 ml. of acetic acid were then added. The water phase was separated and evaporated *in vacuo;* 20 ml. of ethanol was added and the solvent evaporated again; the operation was repeated once more. The final residue was dissolved in 26 ml. of water, and to 13 ml. 1 g. of diphenylhydrazine was added; the solution was then heated in a water bath. When crystals of D-arabinose diphenylhydrazone appeared, heating was stopped, and after four hours in the cold the crystals were collected; yield 54% (calcd. as D-arabinose), m. p. 204–205°.

Degradation of octaacetyllactobiononitrile. (*Sodium ethoxide.*)[60] To 750 ml. of chloroform containing the impure octaacetyllactobiononitrile prepared from 200 g. of lactose, a solution of 15 g. of sodium in 750 ml. of methanol was added; the solution was maintained at a low temperature and was shaken continuously. A jelly-like mass precipitated. When the precipitation was ended, 750 ml. of water and 50 ml. of acetic acid were added. The chloroform layer was separated and the water phase was evaporated *in vacuo* to a thick sirup. This was dissolved in 400 ml. of 96% ethanol, 65 g. of α,α-benzylphenylhydrazine was added and the solution was heated in a water bath for thirty minutes. Assisted by seeding, crystallization of the hydrazone of the 3-(β-D-galactopyranosyl)-D-arabinose was completed in five hours. The crystals were filtered and washed well with 80% ethanol; yield 60–67 g., m. p. 223–225°.

IV. The Diamide Compounds of the Aldoses

1. *Properties of the Diamide Compounds*

With the exception of *N*-acetyl-D-glucofuranosylamine studied by Hockett and Chandler,[47] all the acetylated nitriles of the aldonic acids

(60) G. Zemplén, *Ber.*, **59**, 2402 (1926).

that were degraded by employing ammonia-silver oxide or ammonia in aqueous or alcoholic solution have yielded diacetamide compounds of the aldoses. These diacetamide derivatives are solids that crystallize easily; they are very soluble in water but are much less soluble in methanol or ethanol and are almost insoluble in nonpolar organic solvents. They have been acetylated and benzoylated and some benzylidene derivatives have been prepared (see Table III, page 151). The diacetamides and their derivatives have *aldehydo* structures that can be compared to the thioacetals of the aldoses.

Hydrolysis of the diacetamides is effected by acids. Dilute hydrochloric, sulfuric and nitric acid have been used. An aldose with one carbon atom less than the original nitrile is then liberated from the combination. Only exceptionally can this aldose be isolated in pure condition without transforming it into an insoluble derivative; in these instances, the method has been employed for preparative work. In most cases the sugar has been characterized as an osazone. The aldose, without great purification, has been employed successfully for reduction[37,38] or oxidation experiments.[56–58]

The rate of hydrolysis of D-threose diacetamide by 0.1 N sulfuric acid has been studied by Hockett[38] by titration with iodine, and the rate was found to correspond approximately to that of a unimolecular reaction. The same results were obtained with D-erythrose diacetamide by Hockett and Maynard[13] on following the change of rotatory power during the hydrolysis.

Diacetamides are oxidized by lead tetraacetate, and Hockett and coworkers[61] have studied the behavior of a number of these compounds.

The aldose dipropionamides prepared by Giménez[21] have properties similar to the diacetamides, although their solubility in water is lower.

Dibenzamides of the aldose series were first prepared by Brigl, Mühlschlegel and Schinle[26] and afterward by Restelli de Labriola and Deulofeu.[27] They were highly insoluble in all solvents tried except pyridine.

2. *Experimental Procedures*

Preparation of D-*arabinose diphenylhydrazone from pentaacetyl*-D-*glucononitrile.*[62] To a cold solution of 100 g. of pentaacetyl-D-glucononitrile in 280 ml. of 96% ethanol, 34 g. of silver oxide (from about 50 g. of silver nitrate) dissolved in 500 ml. of 30% ammonia was added. After forty-eight hours the separated crystalline silver cyanide was removed by filtration and the filtrate concentrated *in vacuo* to about one-third of the original volume and filtered. Water was added to 500 ml., and the dissolved

(61) R. C. Hockett, M. T. Dienes, H. G. Fletcher, Jr., and H. E. Ramsden, *J. Am. Chem. Soc.*, **66**, 467 (1944).

(62) C. Neuberg and J. Wohlgemuth, *Z. physiol. Chem.*, **35**, 33 (1902).

silver ion was precipitated with hydrogen sulfide; the excess of hydrogen sulfide was eliminated by boiling the solution. One hundred milliliters of 37% hydrochloric acid was added and the solution was heated for forty-five minutes in a boiling water bath. The hot acid solution was neutralized with a paste of lead carbonate and the solution allowed to cool and filtered. The filtrate was concentrated in an open dish to 250 ml. and the same volume of 96% ethanol added. A new precipitate that was produced was removed by filtration after two hours. The amount of D-arabinose in the filtrate was then determined by reduction of a sample by polarimetric measurement or by precipitation with diphenylhydrazine.

An ethanolic solution containing the calculated amount of diphenylhydrazine was then added to the solution, which was heated for thirty minutes in a water bath. The D-arabinose diphenylhydrazone was filtered after twelve hours and washed with ethanol; yield 62.3 g. (76% from the nitrile employed), m. p. 204–205°. D-Arabinose was obtained in 96% yield from the diphenylhydrazone by treatment with formaldehyde[63] as described by Neuberg.[64]

V. Summary of the Application of the Degradation

D-*Arabinose.* Tetraacetyl-D-arabononitrile was prepared by Deulofeu[58] and degraded to triacetyl-D-erythrose and D-erythrose diacetamide by ammonia-silver oxide. Hockett and Maynard[13] improved the yield of the nitrile and by hydrolysis of D-erythrose diacetamide with 0.6 *N* sulfuric acid obtained D-erythrose as a sirup from which methyl D-erythroside was prepared.

Deulofeu[58] also degraded tetraacetyl-D-arabononitrile with sodium methoxide or by hydrolysis of the acetyl groups with sulfuric acid and separation of the nitrile group with silver carbonate. D-Erythrose was characterized as the phenylosazone.

L-*Arabinose.* Tetraacetyl-L-arabononitrile was prepared by Wohl[1,55] in 60% yield by the action of sodium acetate-acetic anhydride on the oxime. From the nitrile by treatment with ammonia-silver oxide, L-erythrose diacetamide was obtained in 45% yield. Hydrolysis of the diacetamide with 0.3 *N* sulfuric acid gave L-erythrose as a sirup characterized as the phenylosazone. In a similar degradation Deulofeu[46] isolated the tetrose as the benzylphenylhydrazone. Degradation of tetraacetyl-L-arabononitrile was effected by Deulofeu and Selva[57] with sodium methoxide and also by hydrolysis of the acetyl groups with sulfuric acid and removal of the nitrile group with silver carbonate. The L-erythrose was identified in both cases through the phenylosazone.

D-*Xylose.* Maquenne[37] obtained tetraacetyl-D-xylononitrile in 41% yield from the D-xylose employed, by treatment of the oxime with sodium acetate-acetic anhydride. From the nitrile, D-threose diacetamide was obtained in 30% yield by the action of ammonia and it was hydrolyzed

(63) O. Ruff and G. Ollendorf, *Ber.*, **32**, 3234 (1899).
(64) C. Neuberg, *Ber.*, **33**, 2243 (1900).

with 10% sulfuric acid. Tetraacetyl-D-xylononitrile was also prepared by Mendive[4] by the action of pyridine-acetic anhydride on the oxime, the yield being 38%. He also hydrolyzed D-threose diacetamide with 0.3 *N* sulfuric acid and obtained D-threose as a sirup.

Maquenne's method was improved by Hockett,[38] who obtained the nitrile in 48% yield and the D-threose diacetamide in 78% yield; the latter product was hydrolyzed with 0.1 *N* sulfuric acid. Tetraacetyl-D-xylononitrile has been degraded by Deulofeu[58] with sodium methoxide and also by hydrolysis of the acetyl groups with sulfuric acid.

L-*Xylose.* Tetraacetyl-L-xylononitrile was obtained by Deulofeu[65] in 48% yield from the oxime, and from it L-threose diacetamide was prepared in 30% yield by employing ammonia and silver oxide. Hydrolysis of the diacetamide with 0.33 *N* sulfuric acid gave L-threose. A correction of the rotatory power of this tetrose by Hockett[38] led to a reinvestigation of the matter by Hockett, Deulofeu, Sedoff and Mendive,[66] who obtained L-threose diacetamide in 70% yield by using aqua ammonia only, and who studied the hydrolysis with 1 *N* sulfuric acid. In the meantime Iwadare, Fukunaga and Kubota[67] realized the same degradation, employing the method used by Hockett for D-xylose, and isolated L-threose as a sirup.

D-*Fucose* (*Rhodeose*). Votoček[17] obtained tetraacetyl-D-fuconomitrile in 25% yield by treating D-fucose oxime with sodium acetate-acetic anhydride. The nitrile, degraded with ammonia and silver oxide, yielded 5-desoxy-D-lyxose diacetamide in 40% yield. The diacetamide compound was hydrolyzed with 5% hydrochloric acid and the 5-desoxy-D-lyxose was obtained in solution and characterized as the *p*-bromophenylosazone. Hydrolysis of the diacetamide compound with 6 *N* sulfuric acid was realized by Votoček and Valentin[68] and the 5-desoxy-D-lyxose was isolated as a sirup.

With L-fucose oxime Restelli de Labriola and Deulofeu[18] obtained the nitrile in 52% yield by the action of pyridine-acetic anhydride.

L-*Rhamnose.* Tetraacetyl-L-rhamnononitrile was prepared by Fischer[56] in 70% yield from the oxime and sodium acetate-acetic anhydride. The nitrile was transformed by Wohl's method into 5-desoxy-L-arabinose diacetamide in 35% yield. Hydrolysis of the diacetamide with

(65) V. Deulofeu, *J. Chem. Soc.*, 2458 (1929).

(66) R. C. Hockett, V. Deulofeu, A. L. Sedoff and R. J. Mendive, *J. Am. Chem. Soc.*, **60**, 278 (1938); V. Deulofeu, *ibid.*, **58**, 855 (1936).

(67) K. Iwadare, S. Fukunaga and B. Kubota, *Bull. Chem. Soc. Japan*, **12**, 116 (1937).

(68) E. Votoček and F. Valentin, *Collection Czechoslov. Chem. Communs.*, **2**, 36 (1930).

5% hydrochloric acid gave 5-desoxy-L-arabinose in solution; it was characterized as the phenylosazone.

Tetrabenzoyl-L-rhamnononitrile was prepared by Restelli de Labriola and Deulofeu[27] in 91% yield from the oxime with pyridine and benzoyl chloride, and was degraded to 5-desoxy-L-arabinose dibenzamide in very small yield (13%) by treatment with ammonia in ethanol.

D-*Glucose*. Wohl[1] obtained pentaacetyl-D-glucon준nitrile in 40% yield by the action of sodium acetate-acetic anhydride. The nitrile when treated with ammonia-silver oxide gave a 47% yield of D-arabinose diacetamide. Hydrolysis of the diacetamide derivative with 6 N sulfuric acid produced crystalline D-arabinose in 50–60% yield. The process was improved by Neuberg and Wohlgemuth,[62] who obtained D-arabinose in an over-all yield of 34.7% of the D-glucose employed.

Degradation of the nitrile with sodium methoxide was effected by Zemplén and Kiss,[24] and the yield of the pentose was 40.6% of the initial D-glucose. Removal of the acetyl groups with sulfuric acid and of the nitrile group with silver carbonate lowered the yield of D-arabinose to 34%.

A quantitative degradation of the free nitrile was carried out by Wohl and Wollenberg.[30]

Pentapropionyl-D-glucononitrile has been prepared in 40% yield by Giménez[21] from the oxime, using pyridine-propionic anhydride. Degraded with ammonia-silver oxide, it yielded 33% of D-arabinose dipropionamide, and with sodium methoxide, 54% of D-arabinose. Pentabenzoyl-D-glucononitrile was obtained from the oxime with pyridine and benzoyl chloride[27] in 71% yield. Degradation with ethanolic ammonia produced 5-benzoyl-D-arabinose diacetamide in 25% yield. Degradation with sodium methoxide gave 17.6% of D-arabinose.

D-*Mannose*. Pentaacetyl-D-mannononitrile was prepared simultaneously by Wolfrom and Thompson[10] and by Deulofeu[58] in 45% yield. Deulofeu degraded the nitrile with ammonia-silver oxide and obtained D-arabinose diacetamide in 32% yield. Degradation with sodium methoxide produced D-arabinose (yield, 61%); treatment with silver carbonate and acid hydrolysis of the acetyl groups gave 56% of the pentose. Pentabenzoyl-D-mannononitrile has been prepared by Restelli de Labriola and Deulofeu[27] in 93% yield from the oxime and pyridine-benzoylchloride. Whereas degradation with sodium methoxide gave 31% of D-arabinose, the action of ethanolic ammonia produced only a 14% yield of 5-benzoyl-D-arabinose diacetamide.

D-*Galactose*. Wohl and List[59] prepared pentaacetyl-D-galactononitrile from the oxime in 40% yield. Degradation with ammonia-silver oxide gave 40% of D-lyxose diacetamide. Hydrolysis of the diacetamide with

N sulfuric acid produced D-lyxose as a sirup, characterized by its phenylosazone and by oxidation to D-lyxonic acid.

By employing aqua ammonia,[66] the yield of D-lyxose diacetamide from the nitrile was improved to 72%. Degradation of the nitrile with sodium methoxide[54] yielded 30% of D-lyxose, isolated as the *p*-bromophenylhydrazone. Treatment with silver carbonate subsequent to hydrolysis of the acetyl groups resulted in a 22% yield.

Pentapropionyl-D-galactononitrile has been prepared in 52% yield by Giménez[21] from the oxime and pyridine-acetic anhydride. Degradation with ammonia-silver oxide produced D-lyxose diacetamide in 30% yield.

Pentabenzoyl-D-galactononitrile[27] was obtained in 98% yield from the oxime and pyridine-benzoyl chloride; 5-benzoyl-D-lyxose in 22% yield was obtained by degradation with ethanolic ammonia.

D-*Gluco*-D-gulo-*heptose*. Hexaacetyl-D-gluco-D-*gulo*-heptononitrile was obtained by Zemplén and Kiss[24] in 47% yield by treatment of the acetylated amide with phosphorus oxychloride, and also from the oxime and pyridine-acetic anhydride (69% yield)[18] or sodium acetate-acetic anhydride (51% yield).[47] Degradation with sodium methoxide[24] gave D-glucose in 58% yield (isolated as the pentaacetyl derivative); with aqua ammonia[47] a 26% yield of *N*-acetyl-D-glucofuranosylamine was obtained.

D-*Manno*-D-gala-*heptose*. Hexaacetyl-D-manno-D-*gala*-heptononitrile was prepared by Mikšic[22] and by Brigl, Mühlschlegel and Schinle[26] by acetylation of the free nitrile. It was degraded with methanolic ammonia and silver nitrate, giving D-mannose diacetamide in 34% yield.

Hexabenzoyl-D-manno-D-*gala*-heptononitrile was prepared in 89% yield by benzoylation of the free nitrile and degraded in the same way[26] as above to give a yield of 33% D-mannose dibenzamide.

Cellobiose. Zemplén[51] prepared octaacetylcellobiononitrile in 50% yield from the oxime by the action of sodium acetate-acetic anhydride. An acetylated oxime, that Wolfrom and Soltzberg[69] showed is the nonaacetylcellobiose oxime with a cyclic structure, was obtained as a by-product. Octaacetylcellobiononitrile was degraded with sodium methoxide to 3-(β-D-glucopyranosyl)-D-arabinose, isolated as the heptaacetyl derivative in 31–33% yield. The oxime of the new disaccharide was prepared and transformed into heptaacetyl-3-(β-D-glucopyranosyl)-D-arabononitrile, which had a purity of 67%. Degradation by the same method gave 2-(β-D-glucopyranosyl)-D-erythrose, obtained only in solution.

Lactose. Zemplén[60] obtained the octaacetyllactobiononitrile (60–66% purity) from the oxime in a yield of 50%. Degradation with

(69) M. L. Wolfrom and S. Soltzberg, *J. Am. Chem. Soc.*, **58**, 1783 (1936).

sodium methoxide gave a 44–48% yield of 3-(β-D-galactopyranosyl)-D-arabinose, that was isolated through its benzylphenylhydrazone and subsequently crystallized.[70] From its oxime a pure heptaacetyl-3-(β-D-galactopyranosyl)-D-arabononitrile was prepared in 57% yield, a new degradation effected, and 2-(β-D-galactopyranosyl)-D-erythrose obtained as an amorphous powder in a 15–17% yield.

Maltose. An octaacetylmaltobiononitrile of 65% purity has been prepared by Zemplén[71] in about 50% yield. It was degraded to an impure 3-(α-D-glucopyranosyl)-D-arabinose. The oxime was converted to impure heptaacetyl-3-(α-D-glucopyranosyl)-D-arabononitrile which was degraded again to 2-(α-D-glucopyranosyl)-D-erythrose, obtained only in solution.

Melibiose. An octaacetylmelibiononitrile of 64% purity was prepared by Zemplén[72] in about 50% yield, from pure melibiose oxime. It was degraded in a 68–78% yield to 5-(α-D-galactopyranosyl)-D-arabinose, obtained only in solution.

2-(D-*Glucopyranosyl*)-D-*glucopyranose.* This disaccharide was synthesized by Gakhokidze,[73] and from its oxime the octaacetyl nitrile was prepared in 45% yield by the action of sodium acetate-acetic anhydride. He reported that treatment of the nitrile with sodium ethoxide and silver carbonate gave a glucosylpentose, isolated as a crystalline heptaacetyl derivative.

3-(D-*Glucopyranosyl*)-D-*glucopyranose.* This disaccharide, synthesized by Gakhokidze[51a] gave an oxime that without further purification was transformed with a 55.5% yield into octaacetyl-3-(D-glucopyranosyl)-D-gluconontrile. Degradation with sodium methoxide and silver carbonate gave 2-(D-glucopyranosyl)-D-arabinose that was isolated as a crystalline heptaacetyl derivative. The oxime of the 2-(D-glucopyranosyl)-D-arabinose was prepared and transformed with a 49% yield into heptaacetyl-2-(D-glucopyranosyl)-D-arabononitrile, that was submitted to a similar degradation, yielding 1-(D-glucopyranosyl)-D-erythofuranoside isolated as a crystalline hexaacetyl derivative.

NOTE ADDED IN PROOF: Experiments have recently been carried out by Hockett, Deulofeu and Deferrari (private communication) in which tetraacetyl-L-arabononitrile was treated with an alcoholic solution of ammonia containing an excess of N^{15} in the presence of an excess of acetamide containing normal nitrogen. The "L-erythrose diacetamide" isolated from the reaction mixture showed an N^{15} content of such magnitude as to prove that the combined acetamide is derived from interaction of labelled ammonia with combined acetyl groups through some such mechanism as that suggested by Isbell and Frush.[46a] (See page 137.)

(70) G. Zemplén, *Ber.*, **60,** 1309 (1927). (71) G. Zemplén, *Ber.*, **60,** 1555 (1927). (72) G. Zemplén, *Ber.*, **60,** 923 (1927). (73) A. M. Gakhokidze, *J. Gen. Chem.* (U.S.S.R.), **11,** 117 (1941); *Chem. Abstracts*, **35,** 5467 (1941).

VI. Tables

Table I
Nitriles of Aldonic Acids and Acylated Derivatives

Systematic name	*Melting point, °C.*	$[\alpha]_D$	*Rotation solvent*	*References*
A. Pentonic acid nitriles				
Tetraacetyl-D-arabonic	120–121	−3.3°	$CHCl_3$	25, 75
Tetraacetyl-L-arabonic	120–121	+3.4	$CHCl_3$	16, 74
Tetraacetyl-D-ribonic	71–72	+34.4	$CHCl_3$	25
Tetraacetyl-D-xylonic	83	+50.4	$CHCl_3$	76
Tetraacetyl-L-xylonic	81–82	−50.4	$CHCl_3$	65, 66
B. 6-Desoxyhexonic acid nitriles				
Tetraacetyl-L-rhamnonic	70	−4.0	$CHCl_3$	56, 76
Tetrapropionyl-L-rhamnonic	26–28	−6.0	$CHCl_3$	21
Tetrabenzoyl-L-rhamnonic	114	+4.7	$CHCl_3$	27
Tetraacetyl-D-fuconic	177–178	—	—	17
Tetraacetyl-L-fuconic	177–178	−22.4	$CHCl_3$	17, 18
C. Hexonic acid nitriles				
D-Gluconic	120.5	+10.0	H_2O	29
	120.5	+6.0	Pyridine	29
	145	+9.9	H_2O	29
	145	+6.2	Pyridine	29
Pentaacetyl-D-gluconic	83–84	+47.8	$CHCl_3$	10, 24
Pentapropionyl-D-gluconic	68–69	+40.1	$CHCl_3$	21
Pentabenzoyl-D-gluconic	118	+15.1	$CHCl_3$	27
Pentaacetyl-D-mannonic	92–93	−1.8	$CHCl_3$	10, 58
Pentapropionyl-D-mannonic	<0	+5.6	$CHCl_3$	21
Pentabenzoyl-D-mannonic	130	+10.2	$CHCl_3$	27
Pentaacetyl-D-galactonic	138–139	+43.2	$CHCl_3$	16
Pentapropionyl-D-galactonic	59–61	+36.7	$CHCl_3$	21
Pentabenzoyl-D-galactonic	142–144	+9.7	$CHCl_3$	27
Pentaacetyl-D-glucosaminic	126	+20.5	$CHCl_3$	18
N-Methyl-L-glucosaminic	113	−17.5	H_2O	23
N-Methyl-L-glucosaminic hydrochloride	138–140	−28.5	H_2O	23*a*
Pentaacetyl-*N*-methyl-L-glucosaminic	132–134	−38.0	$CHCl_3$	23
Pentaacetyl-*N*-methyl-L-mannosaminic	112–113.5	−27.5	$CHCl_3$	23*a*
D. 7-Desoxyheptonic acid nitriles				
L-Rhamno-L-*manno*-heptonic	145	−23.4	H_2O	22
Pentaacetyl-L-rhamno-L-*manno*-heptonic	85–86	−76.4	$CHCl_3$	22
E. Heptonic acid nitriles				
Hexaacetyl-D-gluco-D-*gulo*-heptonic	113–114	+24.6	$CHCl_3$	18, 24
	85.5–87.5	+24.3	$CHCl_3$	47
D-Manno-D-*gala*-heptonic	121–122	+31.4	H_2O	22
Hexaacetyl-D-manno-D-*gala*-heptonic	124.5–125	+31.4	$CHCl_3$	22
Hexabenzoyl-D-manno-D-*gala*-heptonic	161–162	+30.3	$CHCl_3$	26
Hexaacetyl-D-gala-L-*manno*-heptonic	193–194	+31.7	$CHCl_3$	13
D-Fructoheptonic (fructose cyanohydrin)	110–115	—	—	28

TABLE I (*Continued*)

Systematic name	*Melting point, °C.*	$[\alpha]_D$	*Rotation solvent*	*References*
F. Octonic acid nitriles				
Heptaacetyl-D-gala-L-*gala*-octonic	185	+8.5°	$CHCl_3$	20
G. Bionic acid nitriles				
Heptaacetyl-3-(β-D-galactopyranosyl)-D-arabonic	132	+5	$CHCl_3$	60
Octaacetylcellobionic	132	+34.3	$CHCl_3$	1
Octaacetyl-2-(D-glucosyl)-D-gluconic	149–151	—	—	73
Octaacetyl-3-(D-glucosyl-)-D-gluconic	152–153			51*a*
Heptaacetyl-2-(D-glucosyl-)-D-arabonic	158			51*a*

TABLE II

Acylated Oximes of Aldoses

Systematic name	*Melting point, °C.*	$[\alpha]_D$	*Rotation solvent*	*References*
A. Acylated *aldehydo* oximes				
Pentapropionyl-L-rhamnose	48–50	+4.0°	$CHCl_3$	21
Hexaacetyl-D-glucose	119.5	+46.3	$CHCl_3$	10
	119.5	+41	CH_3OH	10
Hexaacetyl-D-mannose	94	−8.3	$CHCl_3$	76, 77
Hexaacetyl-D-galactose	145–146	+23.6	$CHCl_3$	16
Heptacetyl-D-gala-L-*manno*-heptose	125–126	+38.6	$CHCl_3$	13
Octaacetyl-D-gala-L-*gala*-octose	187–188	+14.9	$CHCl_3$	20
Nonaacetylcellobiose	—	+37	$CHCl_3$	69
B. Acylated cyclic oximes				
Pentaacetyl-D-fucose	115–116	—	—	17
Pentaacetyl-L-fucose	116	+44.9	$CHCl_3$	18
Hexaacetyl-D-glucose	113–115	+7 3	$CHCl_3$	10
	113–115	−1.3	CH_3OH	10
Hexaacetyl-D-galactose	106	−27.5	$CHCl_3$	16
Nonaacetylcellobiose	195–195.5	−8.5	$CHCl_3$	69

(74) Rotatory dispersion in ethyl acetate: W. C. G. Baldwin, M. L. Wolfrom and T. M. Lowry, *J. Chem. Soc.*, 696 (1935).

(75) R. C. Hockett and C. W. Maynard, *J. Am. Chem. Soc.*, **61,** 2111 (1939).

(76) V. Deulofeu, P. Cattaneo and G. Mendivelzua, *J. Chem. Soc.*, 147 (1934).

(77) M. L. Wolfrom and L. W. Georges, *J. Am. Chem. Soc.*, **58,** 1781 (1936).

TABLE III

Diamide Compounds of the Aldoses and Derivatives

Systematic name	*Melting point, °C.*	$[\alpha]_D$	*Rotation solvent*	*References*
A. Tetroses				
D-Erythrose diacetamide	210	—	—	58
L-Erythrose diacetamide	210	−7.9°	H_2O	55
Triacetyl derivative	148	−22.6	$CHCl_3$	78
	148	−35.8	H_2O	46, 78
D-Threose diacetamide	165–167	−10.8	H_2O	38
Triacetyl derivative	179–180	+74.2	$CHCl_3$	38
	176–177	+38	H_2O	79
Tribenzoyl derivative	155–156	+109.7	$CHCl_3$	65
Benzylidene derivative	265	—	—	65
L-Threose diacetamide	165–167	+10.2	H_2O	65
Triacetyl derivative	178–179	−74	$CHCl_3$	65
	178–179	−38.1	H_2O	65
Tribenzoyl derivative	155–156	−110 1	$CHCl_3$	65
Benzylidene derivative	265–266	—	—	65
B. Desoxypentoses				
5-Desoxy-L-arabinose diacetamide	202–204	+19 8	H_2O	78
5-Desoxy-L-arabinose dibenzamide	225–226	−2.1	Pyridine	27
Triacetyl derivative	193	−92.9	$CHCl_3$	27
Tribenzoyl derivative	212	—	—	27
5-Desoxy-L-lyxose diacetamide	233	—	—	17
C. Pentoses				
D-Arabinose diacetamide	187	−9.5	H_2O	1
Tetraacetyl derivative	218–219	+72.5	$CHCl_3$	66
D-Arabinose dipropionamide	177–178	−9.8	H_2O	21
Tetraacetyl derivative	171–172	+67.7	$CHCl_3$	21
5-Benzoyl-D-arabinose dibenzamide	206–208	+8.5	Pyridine	27
Triacetyl derivative	193	+69.0	$CHCl_3$	27
D-Lyxose diacetamide	230–231	−9.2	H_2O	65
D-Lyxose dipropionamide	180–182	−8.5	H_2O	21
5-Benzoyl-D-lyxose dibenzamide	222–224	+36.1	Pyridine	27
Triacetyl derivative	189	+36.2	$CHCl_3$	27
Tetrabenzoyl derivative	227–229	+39.1	$CHCl_3$	27
D. Hexoses				
D-Glucose dibenzamide	202	+1.5	Pyridine	26
D-Mannose diacetamide	219	—	—	26
D-Mannose dibenzamide	226	+3.6	Pyridine	26

(78) E. Restelli de Labriola, Thesis, Facultad de Ciencias Exactas, Fisicas y Naturales, Buenos Aires, 1938.

(79) V. Deulofeu, *J. Am. Chem. Soc.*, **58**, 855 (1936).

WOOD SACCHARIFICATION

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Contents

I. Introduction

The production of sugar from wood waste has claimed the attention of chemists for the last fifty years. It is of interest, both as a process of producing a supplement to agricultural products and also as a means of converting the vast amount of wood waste resulting from lumber manufacture into useful products.

In the manufacture of lumber only half the tree is taken to the mill in the form of sawlogs. The other half remains in the woods as stumps, tops and culls. Of the part taken to the mill, about half results in sawdust, shavings, slabs and edgings. The annual cut of lumber in the United States has ranged from fourteen billion to thirty billion board feet per year. The sawmill waste from this cut is estimated to be about one ton, dry basis, for each thousand board feet of lumber produced. This waste material has already been collected and is available for use at the plant where it originated without any further costs for collection or transportation. This wood waste contains 50–65% carbohydrates in the form of hemicellulose and cellulose. If these carbohydrates were changed into simple sugars, they could be used as a source of carbohydrate feed for cattle, hogs, chickens and other livestock. These sugars would also be available for use in industrial processes as a reducing material for chemical processes, as a thickening material for dye printing and as a starting product for the production of various organic substances. This sugar would also be suitable for fermentation processes for the production of ethyl alcohol, butyl alcohol, acetone, and lactic, butyric, acetic and other acids.

II. History of Wood Saccharification

Processes for the production of sugar from wood are grouped in two classes: (1) the hydrolysis of wood at elevated temperature and pressure with dilute acid (sulfuric or hydrochloric) as a catalyst; and (2) the treatment of wood with strong acid in which it is dissolved and then dilution with water and hydrolysis of the resulting solution.

The dilute-acid process consists of digesting sawdust, shavings or hogged wood with dilute mineral acid at steam pressures of 35 to 200 lb./sq. in. (One atmosphere pressure equals about 14.7 pounds per square inch.) The carbohydrates are converted into a mixture of

pentoses and hexoses. The extent of the conversion depends upon the acid concentration, the length of treatment and the temperature.

The processes in the second class involve the use of concentrated acids, such as 65 to 75% sulfuric acid, 85% phosphoric ac'd, 45% hydrochloric acid and anhydrous hydrofluoric acid. The wood is actually dissolved in the acid and allowed to stand until the cellulose is converted into polysaccharides of low molecular weight. Then the solution is diluted with water and boiled to convert the product into simple sugars. Sulfuric acid has not been used in commercial adaptations of these processes, although it was shown in 1882 by Flechsig that cotton cellulose could be converted almost quantitatively into sugar by the acid.

1. *Dilute-acid Processes*

Records indicate that Braconnot[1] was interested in the dilute-acid method of hydrolysis as early as 1819. Many other workers have made contributions,[2] but it was not until 1898 that an attempt was made to commercialize the process. At that time Simonsen[3] published a paper on the process. His process consisted in treating sawdust for fifteen minutes with four parts of 0.5% sulfuric acid at about 9 atmospheres pressure. He obtained a 6% yield of sugar, based on dry wood.

A. C. Classen[4] used sulfur dioxide as the hydrolytic agent, and experimental plants were built in France to determine the suitability of this process. In 1903 patent rights were sold to an American company, which built an experimental plant at Highland Park, Illinois. Later this company erected a plant at Hattiesburg, Mississippi, to operate on sawmill waste of longleaf pine. Because of mechanical difficulties and a failure to understand the principles involved,[5] the plant closed. Another plant was built at Port Hadlock, Washington, which used the sulfur dioxide process, but it, too, failed after a short time.

M. F. Ewen and G. H. Tomlinson, who had been associated with the Hattiesburg project, continued their experimentations and took out U. S. Patent No. 938,308, which covered the use of dilute sulfuric acid as

(1) H. Braconnot, *Gilbert's Annalen der Physik*, **63**, 348 (1819); *Ann chim. phys.*, **12**, 172 (1819).

(2) References from F. W. Kressman, *U. S. Dept. Agr. Bull.* 983 (1922): J. E. Arnould, *Compt. rend.*, **39**, 807 (1854); E. Fleschsig, *Z. physik. Chem.*, **7**, 523 (1883); E. Kern, *J. Landw.*, **24**, 19 (1876); G. Kuhn, L. Aronstein and H. Schulze, *ibid.*, **10**, 304 (1862); J. B. Lindsey and B. Tollens, *Ann.*, **267**, 341 (1892); Melsen, *Genie industriel* (1855); A. Payen, *Compt. rend.*, **48**, 210 (1859); T. J. Pelouze, *Dingler's J.*, **15**, 394 (1859).

(3) E. Simonsen, *Z. angew. Chem.*, **195**, 962, 1007 (1898).

(4) A. C. Classen, German Pats. 111,868, 118,542, 118,543, 118,544.

(5) R. Ruttan, *J. Soc. Chem. Ind. (London)*, **28**, 1290 (1909).

the hydrolytic agent under conditions similar to those used by Simonsen. A plant using their process was built at Georgetown, South Carolina, which operated from about 1913 to 1923.

Another plant using dilute sulfuric acid for hydrolysis was built at Fullerton, Louisiana, in 1916 to produce daily 5,000 gallons of 188-proof alcohol. The Forest Products Laboratory, Madison, Wisconsin, assisted in the development and pilot plant work of both the Georgetown, and the Fullerton plants. The results of the work at these plants and in a Forest Products Laboratory pilot plant have been described by F. W. Kressman in U. S. Department of Agriculture Bulletin No. 983 (1922). This bulletin describes pilot plant investigations on the following variables: (1) temperature and pressure of digestion; (2) length of time of digestion; (3) ratio of acid and water to dry sawdust; (4) concentration of acid; (5) size of sawdust or hogged wood; (6) effect of adding acid after preliminary heating of wood; (7) effect of bark, tannins and other wood ingredients; (8) effect of special chemical treatments; (9) yields from different species of wood; (10) variations in fermentability due to different conditions; and (11) steam consumption.

An enamel-lined rotary digester, which was heated by direct steam, was used for the hydrolysis in these investigations. The sugars produced by the hydrolysis were extracted from chips in an extraction battery. The sugar solution was neutralized with lime, filtered and then prepared for fermentation.

Variations in temperature and pressure were conducted in two series, one at a water-to-wood ratio of 4:1 with an acid-to-wood ratio of 1.8:100, and the other at water-to-wood ratio of 1.25:1 with an acid-to-wood ratio of 2.5:100. In each series the yield of sugar produced in fifteen minutes increased from 14% at 4 atmospheres pressure to 23% at 7.5 atmospheres. Higher pressures caused a decrease in sugar yield. Maximum yields of fermentable sugars were obtained with 1.25 parts water, containing 2.0% sulfuric acid, in 20 minutes at 7.5 atmospheres. Softwoods and hardwoods gave about the same yield of reducing sugar. The sugars from softwood were about 70% fermentable, and those from hardwood about 30% fermentable. The yield of sugar ranged from 16 to 26%. A mixture of sawdust and small chips gave better results than sawdust alone. These developments became known as the American process.

Experiments were continued by E. C. Sherrard and his associates at the Forest Products Laboratory. Special methods of treatment[6] produced sufficient sugar from western larch to yield 40 gallons of alcohol

(6) E. C. Sherrard, *Ind. Eng. Chem.*, **14**, 948 (1922).

per ton of dry wood. Tests with other acids[7] under various conditions indicated that, provided sufficient ionizable acid were present, the yield of sugar in one treatment would be about the same. From 1923 to 1927, Sherrard and Davidson[8] of the U. S. Forest Products Laboratory conducted experiments on successive treatments of spruce wood with dilute sulfuric acid. Yields of sugar were increased by the process to about 40% of the weight of the wood.

While these developments were in progress in America, similar progress was being made in Germany and France.[9,10] Developments in Germany were described in a review by Heuser,[11] where more consideration was given because of shortages of carbohydrates. The experiments on successive treatments with acid were continued by Lüers[12] and Scholler[13] and resulted in the process known as the Scholler process. The conditions of temperature, time and acid concentration were the same as those used previously by Sherrard and Davidson. Scholler's contribution was the use of a stationary digester instead of a rotary digester. In Scholler's experiments hydrolysis was continued until all the cellulose was hydrolyzed. This procedure yielded concentrations of 3 to 3.5% reducing sugar, and yields were 45 to 55% of the weight of the dry wood. A report of the most recent progress on the Scholler process was given by special investigators[14] who were sent to Germany at the close of the war to learn about German scientific developments.

Patent rights to the Scholler process were sold in the United States

(7) E. C. Sherrard and W. H. Gauger, *Ind. Eng. Chem.*, **15**, 63, 1164 (1923); E. C. Sherrard and G. W. Blanco, *Ind. Eng. Chem.*, **15**, 611 (1923).

(8) E. C. Sherrard and P. B. Davidson, "Hydrolysis of Wood by Successive Hydrolyses of Spruce Wood," presented before Am. Chem. Soc. Meeting, Detroit (1927).

(9) G. Meunier, *Compt. rend.*, **174**, 468 (1922).

(10) M. Junien, *Bull. assoc. chim. sucr. dist.*, **49**, 153 (1932).

(11) E. Heuser, *Cellulosechemie*, **1**, 41 (1920).

(12) H. Lüers, *Angew. Chemie*, **43**, 455 (1930), **45**, 369 (1932); *Holz Roh- u. Werkstoff*, **1**, 35 (1937); *Z. Spiritusind.*, **60**, 7 (1937); *Holz Roh- u. Werkstoff*, **1**, 342 (1938).

(13) H. Scholler, French Pat. 706,678, (1930); *Z. Spiritusind.*, **55**, 94 (1932); *Wochbl. Papierfabrib.*, May 1934; *Zellstoff-Faser*, **32**, 64 (1935); French Pat. 777,824 (1935); *Chem. Z.*, **60**, 293 (1936); French Pat. 799,358 (1936); *Rundschau deut. Tech.*, March 24 (1938); *Tech. Bur. Percola*, Mar. 1 (1939), June 1 (1939), July 26 (1940); *Chim. ind. agr. biol.*, **15**, 195 (1939); *Chem. Z.*, **63**, 737, 752 (1939); U. S. Pats. 1,641,-771 (1927); 1,890,304 (1932); 1,990,097 (1935); 2,083,347 (1935); 2,083,348 (1937); 2,086,963 (1937); 2,088,977 (1937); 2,108,567 (1938); 2,123,211 (1938); 2,123,212 (1938); 2,188,192 (1940); 2,188,193 (1940).

(14) J. F. Saeman, E. G. Locke and G. K. Dickerman, FIAT Report 499, "Production of Wood Sugar in Germany and its Conversion to Yeast and Alcohol," Nov. 14, 1945.

to the Cliffs Dow Chemical Company and to the Tennessee Eastman Company; both concerns made significant improvements in the process. Their rights were abandoned because they felt that sugar produced by the process could not compete with the low price of blackstrap molasses then prevailing.

2. *Dilute-acid Process plus Production of Plastic Powder*

Sherrard and coworkers[15–18] concluded that a process of hydrolysis that would not only produce sugar but also a valuable residue, would be necessary to make wood hydrolysis profitable. In the period 1923 to 1940 their hydrolysis experiments were directed toward the production of a residue suitable as a plastic molding powder or sheet for laminated molding. They found that hardwoods produced the best residue for this purpose. This procedure was later developed for a rapid, continuous hydrolysis,[19–21] with conditions varied so as to give products with differing degrees of plasticity, strength and moisture absorption. Hydrolysis by the continuous process was carried out at temperatures of 150° to 187°C. for 1.8 to 8.6 minutes with 0.4 to 3.0% sulfuric acid. Because of the need for a slurry that could be pumped, the dilute-acid-to-wood ratio was 8 to 10 parts to 1. This gave sugar solutions too dilute to be of value. Recycling of the solutions to build up the concentration caused a destruction of the sugar and the formation of furfural and other sugar decomposition products.

Because of the need for a source of sugar for the production of alcohol, in 1943 the Office of Production Research and Development of the War Production Board provided funds and requested the Forest Products Laboratory at Madison, in cooperation with the Cliffs Dow Chemical Company of Marquette, Michigan, to investigate the hydrolysis of various American woods by successive treatments with dilute sulfuric acid. The results of this investigation are described in U. S. Forest Products Laboratory Report No. R1446. Further investigations are discussed on pages 166 to 178.

Continuing the work that A. C. Classen conducted in 1903 to 1910,

(15) E. C. Sherrard and E. Beglinger, U. S. Pat. 1,932,255 (1933).

(16) E. C. Sherrard, E. Beglinger, and J. P. Hohf, U. S. Pat. 2,130,783 (1938).

(17) E. C. Sherrard, E. Beglinger, J. P. Hohf and E. Bateman, U. S. Pat. 2,153,316 (1938).

(18) E. C. Sherrard, E. Beglinger, and J. P. Hohf, "Wood Plastics as Developed at the Forest Products Laboratory and Their Future Importance," *Forest Products Lab. Rep.* No. R1209, June 1939.

(19) E. T. Olson, R. Katzen, and R. H. Plow, U. S. Pat. 2,156,159 (1939).

(20) E. T. Olson and R. H. Plow, U. S. Pat. 2,156,160 (1939).

(21) R. Katzen and D. F. Othmer, *Ind. Eng. Chem.*, **34**, 314 (1942).

which used sulfur dioxide as the hydrolytic agent, but applying the improvements made by Sherrard[8] and Scholler,[13] Ant-Wuorinen[22] developed a method for producing high yields of sugar. He claims as a special advantage for his method that there is less sugar decomposition than when sulfuric acid is used.

Dilute hydrochloric acid has also been used to hydrolyze wood. Early work is credited to Cohoe.[23] This method was reinvestigated by Miller and Swanson,[24] who subjected wood to successive treatments with 0.75 and 3.0% hydrochloric acid at 96°C., and by Faucounau[25] at higher temperatures.

3. *Concentrated-acid Processes*

Strong sulfuric acid actually dissolves cellulose and promotes a type of hydrolysis that is different from that occurring with dilute acid. Fleschsig[26] in 1883 showed that cotton is converted almost quantitatively into sugar if it is dissolved in concentrated sulfuric acid and then diluted and heated. This procedure has been made the basis for a method for lignin determination.[27] The process has the disadvantages that the amount of acid required is large and that no method has been found for its recovery. Recent work at the Northern Regional Research Laboratory, Peoria, Illinois,[28] on agricultural wastes has effected improvements over previous processes that may make concentrated sulfuric acid adaptable for commercial use in cellulose saccharification.

4. *Bergius-Willstätter Process*

The use of fuming hydrochloric acid has received more attention. Willstätter and Zechmeister[29] found that cellulose dissolved readily in 42–45% hydrochloric acid. Their discovery was made the basis for the Willstätter method for isolating lignin. Wohl and Krull[30] obtained a 60.9% yield of apparent D-glucose when dry pine shavings were dissolved in fuming hydrochloric acid and allowed to stand for five hours at 20°C.

(22) O. Ant-Wuorinen, *Svensk Papperstidn.*, **45**, 149 (1942); *Finnish Paper Timber J.*, **24**, Special No. 7a, 6 (1942); *Chem. Tech. Berlin, Chem. Appa.*, **15**, 253 (1942); *Suomen Kemistilehti*, **15A**, 31 (1942).

(23) W. P. Cohoe, U. S. Pats. 985,725 (1911) and 985,728 (1911).

(24) R. N. Miller and W. H. Swanson, *Ind. Eng. Chem.*, **17**, 843 (1925).

(25) L. Faucounau, *Bull. inst. pin*, 11–23, 70–88 (1934).

(26) E. Fleschsig, *Z. physik. Chem.*, **7**, 523 (1883).

(27) G. J. Ritter, R. M. Seborg, and R. L. Mitchell, *Ind. Eng. Chem., Anal. Ed.*, **4**, 202 (1932).

(28) J. W. Dunning and E. C. Lathrop, *Ind. Eng. Chem.*, **37**, 24 (1945).

(29) R. Willstätter and L. Zechmeister, *Ber.*, **46**, 2401 (1913).

(30) A. Wohl and H. Krull, *Cellulosechemie*, **2**, 1 (1921).

The acid was then removed by vacuum distillation. The residue was dissolved in water and boiled for eight hours. Cellulose under the same conditions gave 97% yields of sugar. Hägglund[31] made a study of the amount of acid required and found that most of it could be recovered and that 76% of the wood could be recovered as a 30% solution of sugar polymers that yielded pentose, D-glucose and isomaltose upon subsequent hydrolysis. A similar procedure came to be known as the Prodor process[32] in France. Ormandy[33] described the commercialization of the hydrochloric acid process that became known as the Rheinau process. Many variations have been made in the process[34–37] from 1927 to 1939. Oil as a heating medium was suggested by Tanchyna and Fanto.[38] The products of the conversion by strong acid were found to be cellotriose, cellotetraose and cellohexaose.[39] The crude sugar products, after dilution and secondary hydrolysis when pine wood was used, were found to be 56% D-glucose, 18% D-mannose, 2.5% D-galactose, 1.5% D-fructose, 7% D-xylose, 2% lignin and 11% undetermined substances.[40] Conditions were worked out for recovery and reuse of most of the hydrochloric acid.[41] It was suggested that the products might be used for cattle feed.[42,43]

Many of these findings were made use of in a second plant that was erected at Regensburg, Germany,[14] at a cost of about eight million dollars. This plant had a capacity of about 130 tons of wood per day. Sugar yields of about 60% were claimed.

Hydrofluoric,[44,45] formic[46–48] and other acids[49] in concentrated solu-

(31) E. Hägglund, *Svensk Kem. Tid.*, **35**, 2 (1923).
(32) G. Vernet, *Chimie et industrie*, Special No. 654, May (1923).
(33) W. R. Ormandy, *J. Soc. Chem. Ind. (London)*, **45**, 267 (1926).
(34) Y. Kauko and Erma Otto, *Ann. Acad. Sci. Fennicae*, **26A**, 24 (1927).
(35) P. Leone and A. Noera, *Ann. chim. applicata*, **18**, 205 (1928).
(36) E. Hunter, *J. Chem. Soc.*, 2643 (1928).
(37) M. Naphthali, *Z. angew. Chem.*, **43**, 215 (1930).
(38) J. V. Tanchyna and H. M. Fanto, *Chem. Listy*, **24**, 105 (1930).
(39) L. Zechmeister and G. Toch, *Ber.*, **64**, 854 (1931).
(40) E. Hägglund, *Tek. Tid. Uppl. C. Kemi*, **63**, 65 (1933).
(41) F. Bergius, *Chem. Trade J.*, **93**, 356 (1933).
(42) F. Bergius, *Atti congr. intern. chim. 10th Congr. Rome, 1938*, **1**, 116 (1939).
(43) E. Hägglund, *Svensk Papperstidn.*, **46**, 123 (1942).
(44) K. Fredenhagen and G. Cadenback, *Angew. Chem.*, **46**, 113 (1938).
(45) H. Lüers, *Holz Roh- u. Werkstoff*, **1**, 342 (1938); *Chem. Abstracts*, **33**, 1492 (1939).
(46) E. Heuser and W. Schott, *Cellulosechemie*, **6**, 10 (1925).
(47) L. Vol-Rabinovich, *Lesokhim. Prom.*, **2**, 1 (1933).
(48) H. M. Guinot, *Chimie industrie*, **46**, 283 (1941); *Chem. Abstracts*, **36**, 6013 (1941).
(49) Franz Schutz, *Cellulosechemie*, **18**, 76 (1940).

tion have been used for the hydrolysis of cellulose. These, however, have the same disadvantages as other strong acids.

III. Composition of Wood as It Influences Hydrolysis

Wood is composed of two different types of carbohydrate materials, which differ greatly in their ease of hydrolysis. They are hemicellulose, which is easily hydrolyzed, and alpha cellulose, which hydrolyzes slowly.

1. *Hemicellulose*

The hemicellulose of wood differs in its composition according to the type of wood. In general, the hemicellulose of softwoods, upon hydrolysis, gives products that contain about equal parts of fermentable and nonfermentable sugars; while that of hardwoods gives sugars that are about 25% fermentable. The term "hemicellulose" is also applied to the material in wood that is dissolved in cold alkali. Upon hydrolysis, sugars such as xylose, arabinose, mannose, galactose and glucose are obtained in addition to uronic acids and other products related to carbohydrates. Hardwoods give products high in xylose. Some of the softwoods, such as larch, give products high in galactose. Hemicellulose also contains acetyl and methoxyl groups,[50] which are liberated from it during the hydrolysis of wood. Table I gives the composition of spruce hemicellulose as determined by Kurth and Ritter.[51]

In studies on the hydrolysis of wood,[52,53] the hemicellulose that it contains hydrolyzes at a rate so rapid that it is not measurable in the ordinary cellulose hydrolysis procedure. In most calculations hydrolysis is assumed to be instantaneous. Hemicellulose is made up of a number of substances of varying ease of hydrolysis. A portion may be cleaved from wood and may become water-soluble by the hydrolytic action of water. Material hydrolyzed in this manner is not converted completely to simple sugars and has a low fermentability unless subjected to a secondary hydrolysis. Because of the low temperatures and the failure to control the acid concentration in the initial stage of hydrolysis in the Scholler process as practiced in Germany, it was necessary to subject the first liquors obtained to a secondary hydrolysis. The greater ease of hydrolysis of hemicellulose results in high initial concentrations of sugar and, if properly controlled, it aids in maintaining higher average concentrations of reducing sugars[52] in experiments using multistage or continuous treatment.

(50) R. L. Mitchell and G. J. Ritter, *J. Am. Chem. Soc.*, **62,** 1958 (1940).

(51) E. F. Kurth and G. J. Ritter, *J. Am. Chem. Soc.*, **56,** 2720 (1934).

(52) E. E. Harris, E. Beglinger, G. J. Hajny and E. C. Sherrard, *Ind. Eng. Chem.*, **37,** 12 (1945).

(53) J. F. Saeman, *Ind. Eng. Chem.*, **37,** 43 (1945).

TABLE I

Percentage Composition of Easily Hydrolyzable Hemicelluloses in Spruce Holocellulose (Kurth and Ritter[51])

Hemicellulose[a]	*Upon basis of hydrolyzed material*	*Upon basis of wood*
Mannose anhydride	17.7	1.8
Glucose anhydride	8.0	0.8
Galactose anhydride	7.8	0.8
Arabinose anhydride	12.5	1.3
Xylose anhydride	20.9	2.2
Methoxyl	3.2	0.3
Uronic acid anhydride (glucuronic acid)	14.6	1.5
Volatile acids (formyl and acetyl groups)	8.0	0.8
Undetermined	7.3	0.8
Total	*100.0*	*10.3*

[a] Total material dissolved, 10.3% of the extractive-free wood.

2. *Stable Cellulose*

The more stable carbohydrate constituent of wood is assumed to be almost pure alpha cellulose. This is shown by the fact that 94–96% of the reducing materials, obtained in the later stages of hydrolysis, is fermentable. High temperatures and longer periods of time are required for the hydrolysis of the stable cellulose when dilute acid is used. If strong acid (72% sulfuric, 45% hydrochloric, 85% phosphoric) is used, the cellulose dissolves and then is converted into smaller units. Both of these procedures have been studied by many experimenters.

3. *Rates of Hydrolysis of Stable Cellulose*

The term hydrocellulose[54] has been assigned to the hydrolytic products intermediate between cellulose and D-glucose. When cellulose is subjected to the action of acid for even brief periods, there is a change in its tensile strength,[55] copper number and viscosity.

More recently, a number of investigators have studied the rates of hydrolysis of cellulose in strong acid at low temperatures. In most of this work, fuming hydrochloric acid (41–45%) or 50% or stronger sulfuric acid was used. The work of Freudenberg is especially note-

(54) A. Girard, *Compt. rend.*, **81**, 1105 (1875).

(55) C. Birtwell, D. A. Clibbens and A. Geake, *J. Textile Inst.*, **17**, 145 (1926), **19**, 349 (1928).

worthy.[56–59] He found that cellulose first dissolved in the acid to form a viscous solution, from which it could then be precipitated by dilution with cold water. On standing, the solution lost viscosity, and after a period of time no precipitate was formed upon dilution. Staudinger[60] and other workers believe that this change in viscosity is a measure of the change in molecular weight of the cellulose.

Wolfrom and coworkers[61–63] studied the changes that occurred in cellulose in strong hydrochloric acid under conditions that gave an ethyl mercaptan derivative of the hydrolytic product. D-Glucose diethyl mercaptal derivatives did not appear until the hydrolysis was two-thirds complete. The extent of hydrolysis was determined by the number of sulfur groups introduced.

The importance of time, temperature and acid concentration in the hydrolysis of cellulose with dilute acid was recognized by early investigators and applied in the investigations of Simonsen[3] in 1898. Further study was made by Kressman and reported in U. S. Department of Agriculture Bulletin No. 983. Reviews of the quantitative aspects have been made by Doree.[64] Lüers[65] pointed out that the conversion of cellulose dextrin to D-glucose by dilute sulfuric acid was a monomolecular reaction. The constants of the hydrolysis of wood cellulose have been determined by Saeman.[53] The reaction rate (k) was found to be expressed by the following equation:

$$k = HC_s{}^M e^{-\Delta H_a/RT}$$

where H_a is the energy of activation in calories, C_s the concentration of the sulfuric acid in percent, M the slope of the line obtained by plotting log k against the log of the acid concentration and H a constant.

Values determined by Saeman for the hydrolysis of Douglas fir are shown in Table II.

Further work[66] led to the conclusion, since the rates of hydrolysis did

(56) K. Freudenberg, *Trans. Faraday Soc.*, **32,** 74 (1936).

(57) K. Freudenberg, W. Kuhn, W. Durr, F. Bolz and G. Steinbrunn, *Ber.*, **63B,** 1510 (1930).

(58) K. Freudenberg and K. Soff, *Ber.*, **66A,** 19 (1933).

(59) K. Freudenberg and G. Blomquist, *Ber.*, **68B,** 2070 (1935).

(60) H. Staudinger, *Trans. Faraday Soc.*, **29,** 18 (1933).

(61) M. L. Wolfrom and L. W. Georges, *J. Am. Chem. Soc.*, **59,** 282 (1937).

(62) M. L. Wolfrom and J. C. Sowden, *J. Am. Chem. Soc.*, **60,** 3009 (1938).

(63) M. L. Wolfrom, L. W. Georges and J. C. Sowden, *J. Am. Chem. Soc.*, **60,** 1026 (1938).

(64) C. Doree, "Methods of Cellulose Chemistry"; D. Van Nostrand Co., New York, pp. 144–180 (1933).

(65) H. Lüers, *Z. angew. Chem.*, **43,** 455 (1930).

(66) J. F. Saeman and E. E. Harris, presented before the High Polymer Forum, Am. Chem. Soc. meeting, Chicago (1946).

not change as the reaction progressed, that the material being hydrolyzed represented a rather uniform substance. It was assumed that high-molecular-weight cellulose was first converted rapidly into a lower-molecular-weight material that contained about 150 D-glucose units. The conversion of this material into soluble products was slow and measurable, while the conversion of soluble products into D-glucose was rapid and not a limiting factor.

TABLE II
Hydrolysis of Douglas Fir (Saeman[58])

Temperature, °C.	*Sulfuric acid concentration, %*	*First-order reaction constant, $k(min.)^{-1}$*	*Half-life of resistant cellulose, min.*
170	0.4	0.00355	195.0
	0.8	.00886	78.2
	1 6	.02220	31.2
180	0.4	.00950	69.6
	0.8	.02580	26.8
	1.6	.06640	10.4
190	0.4	.02990	23.2
	0.8	.07250	9.56
	1.6	.18300	3.78

IV. DECOMPOSITION OF PRODUCTS OF HYDROLYSIS

There appears to be little evidence of decomposition of unhydrolyzed cellulose in acid solution, but the products of hydrolysis decompose at almost the same rate that cellulose is hydrolyzed by dilute acid. The use of high concentrations of acid at low temperatures, as in the hydrolysis with fuming hydrochloric acid, favors hydrolysis over sugar decomposition and, therefore, higher yields of sugar are obtained. For that reason hydrolysis in concentrated acid is made the basis for determination of the carbohydrate content of wood.[67]

The decomposition of the sugars in the hydrolyzate, when subjected to continued action of acid, was recognized by early investigators. It was found also that pentoses decomposed more rapidly than hexoses. In the work by Kressman[68] this decomposition was studied and its control was employed as a means of increasing the amount of fermentable sugar in the hydrolyzate. In this work it was found that the maximum

(67) J. F. Saeman, J. L. Bubl, and E. E. Harris, *Ind. Eng. Chem., Anal. Ed.*, **17**, 35 (1945).

(68) F. W. Kressman, U. S. Dept. Agr. Bull. 938, (1922).

TABLE III
Decomposition of Various Sugars in 0.8% Sulfuric Acid at 180°C.[69]

Sugar	First-order reaction rate $k(min.^{-1})$	Half-life, min.
D-Glucose	0.0242	28.6
D-Galactose	0.0263	26.4
D-Mannose	0.0358	19.4
L-Arabinose	0.0421	16.4
D-Xylose	0.0720	9.6

yield of sugar in one treatment resulted after a short hydrolysis, and that further heating resulted in the decomposition of the sugar as fast as it was formed. If the reaction were continued long enough, all the cellulose would be hydrolyzed and the resulting sugar decomposed.

The rates of decomposition[69] of various sugars are shown in Table III. A comparison of Table III with Table II shows that the half-life

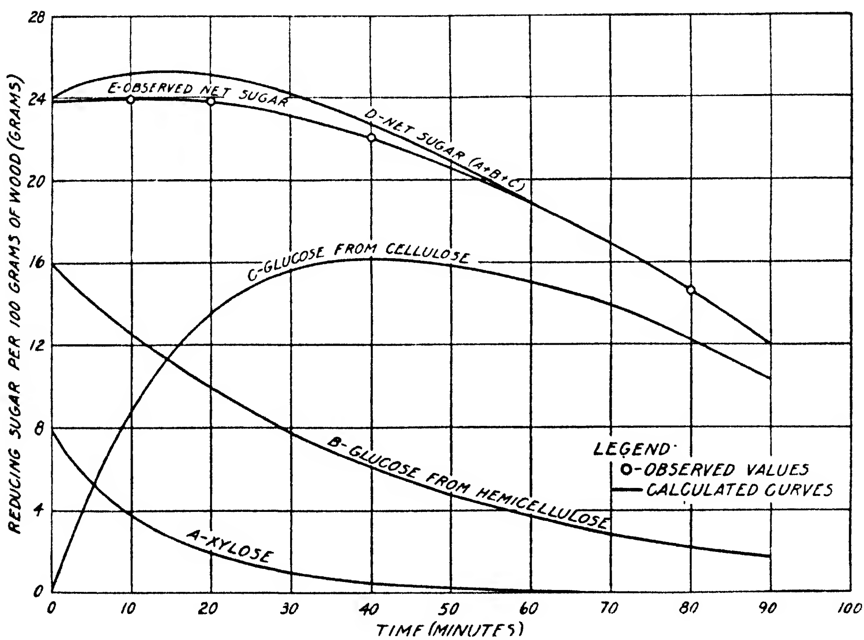

FIG. 1.—The hydrolysis of 30-mesh Douglas fir at 180°C. in 0.8% sulfuric acid with a 10:1 liquid-solid ratio.[53]

(69) J. F. Saeman, E. E. Harris and A. A. Kline, *Ind. Eng. Chem., Anal. Ed.*, **17**, 95 (1945).

of D-glucose is slightly greater than the half-life of cellulose under the same conditions, while D-xylose and other wood sugars have a shorter half-life. This accounts for the increase in fermentable sugars when more drastic conditions are used.

The curves in Fig. 1 by Saeman[53] show the production of sugars by hydrolysis of the carbohydrate and the decomposition of the sugars in the presence of an acid.

These facts show why the attempt to produce high yields of sugars in a one-stage hydrolysis with dilute acid failed. The first published report of hydrolysis of wood cellulose in multiple treatments was by Meunier in 1923. His findings have been made the basis for the production of motor fuels in France.[70] This report was followed by that of Sherrard and Davidson,[8] who increased the treatments still further; and by that of Lüers,[71,72] who published the work of two of his coworkers, Meiler and Scholler, showing that increasing the number of treatments to eighteen or twenty with removal of the sugars between each treatment gave the highest yield.

V. Multistage Process of Hydrolysis

The multistage treatment with dilute acid has been the most popular in Germany and is known as the Scholler process. Three plants were built in Germany.[14] The Tornesch plant, built in 1934, had a capacity of about 25 tons of wood or 1,250 U. S. gallons of 95% alcohol per day; the Dessau plant, completed in 1936, had a capacity of about 60 tons of wood waste per day; and the Holzminden plant, built in 1937, also had a capacity of 60 tons of dry wood waste per day. In all these plants, operation was by manual control, which required a large number of workers. The operating schedule was divided as follows: filling, 1.5 to 2 hours; heating, 0.5 to 1 hour; percolation, 12 to 18 hours. The acid concentration used was 0.8%, with higher concentration for the first treatment. Other Scholler plants were built at Ems, Switzerland, and in Korea, and another was under construction in Italy in 1942.

VI. Pilot Plant Experiments on Wood Hydrolysis

Research for improvements in the process of hydrolysis with dilute acid were discontinued in Germany at an early date. The following

(70) C. Berthelot, "Combustibles et Lubrifiants de Remplacement," Hermann et Cie, Paris (1943).

(71) H. Lüers, *Z. angew. Chem.* **43**, 455 (1930).

(72) H. Lüers, *Z. angew. Chem.* **45**, 369 (1932).

pages describe some of the recent findings of the staff at the Forest Products Laboratory.[52,53,67,69,73–77]

1. *Equipment for Hydrolysis of Wood*

Equipment for hydrolysis with dilute sulfuric acid presents many problems; first, because of the corrosive action of the acid; second, because of the high pressures required; and third, because of tarry material that precipitates from the sugar solutions. A great variety of acid-resistant metals have been tested for the various parts of the equipment. Copper–silicon bronze or Monel metal has been found superior to other materials tested. When these metals were used, the greatest corrosion was found to occur in the pipe lines after the mixing of the acid with the water and before the acid came into contact with the wood or sugar solutions. Another source of corrosion was the volatile organic acids, which caused corrosion in vent valves and in other areas where parts may be exposed to volatile acid vapors in the absence of sugar solutions. The wood–sugar solutions contained corrosion-inhibiting materials sufficient to permit the use of ordinary brass valves in all hydrolyzate lines.

Tarry material was carried along with the wood hydrolyzate and deposited on cooling surfaces or in parts where flow was restricted. For that reason it was necessary to use gate or lubricated plug valves for all hydrolyzate lines. Heat exchangers for conserving heat in the liquor could not be used because of the fouling effect of the tar that precipitated on the tubes.

The pilot plant equipment used at the Forest Products Laboratory for wood hydrolysis is shown in Fig. 2. The chipper is a wood hog of the type used for producing hogged wood for fuel. Water is pumped by an iron triplex with speed control and is heated in iron pipe by discharging steam into it. Strong sulfuric acid (66° Baumé) is pumped by an iron-proportioning pump through iron pipe to the point of injection into the hot water. Several different materials, including glass, quartz and acid-resistant alloys, have been tested for this injector. A lead core project-

(73) E. E. Harris, "Hydrolysis of Wood by Percolation with Dilute Sulfuric Acid and the Fermentation of the Resulting Wood Liquors," *Forest Products Lab. Rept.* No. R1446, March, 1944. Report to Office of Production Research and Development of the War Production Board.

(74) R. H. Plow, J. F. Saeman, H. D. Turner and E. C. Sherrard, *Ind. Eng. Chem.*, **37**, 36 (1945).

(75) E. E. Harris, "Saccharification of Wood," *Forest Products Lab. Rept.* No. R1475, March, 1945.

(76) E. E. Harris and E. Beglinger, *Ind. Eng. Chem.*, **38**, 890 (1946).

(77) J. F. Saeman, E. E. Harris and A. A. Kline, presented before Am. Chem. Soc., Chicago, September, 1946.

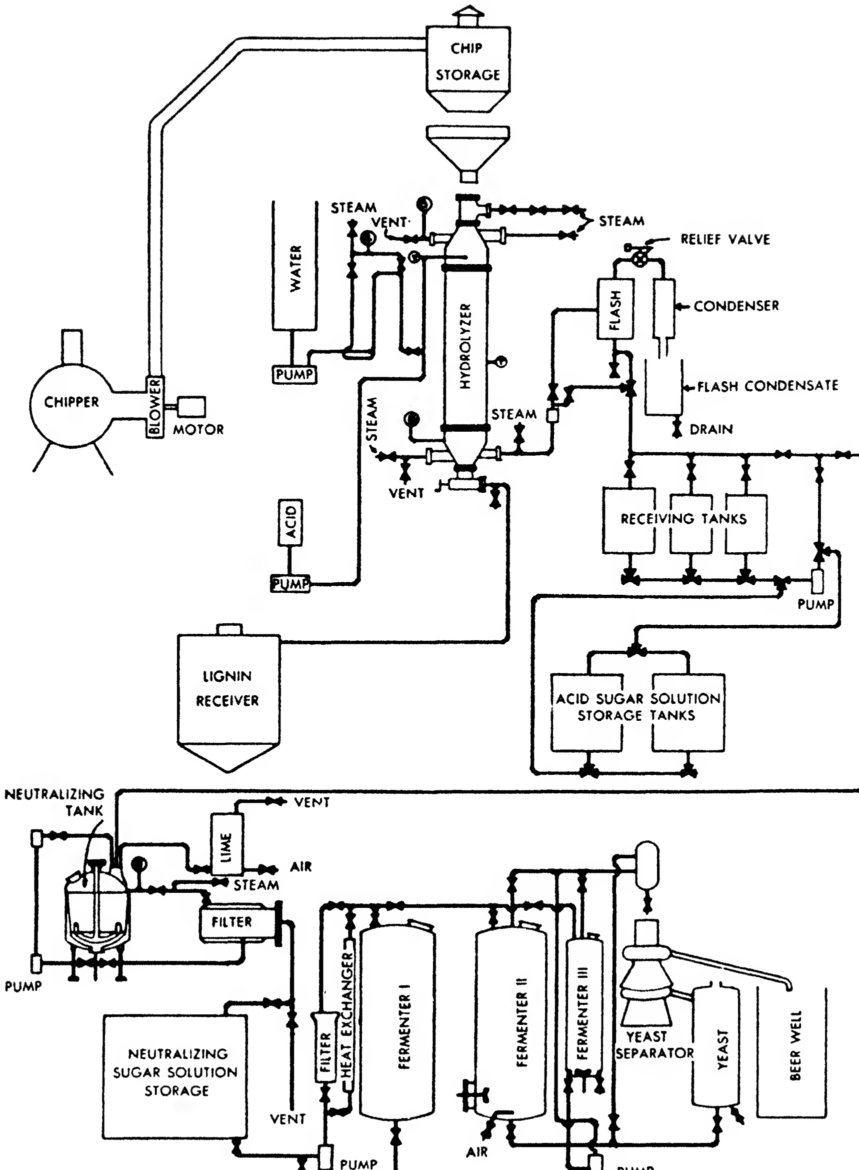

FIG. 2.—Equipment for saccharification of wood and fermentation of wood-sugar solutions.[76]

ing some distance into the water stream has been found the most suitable. The hot dilute acid flows through a short length of copper–silicon alloy to the top of the digester and is introduced through two openings into the top of the copper–silicon–bronze hydrolyzer. The acid hydrolyzate is removed from the bottom and flows through copper pipe and copper flashing equipment to storage or neutralizing equipment. Lignin is discharged at the end of the hydrolysis through an iron pipe into an iron cyclone receiver. The inhibiting action of the lignin and of the residual sugars in the lignin protect the iron from excessive corrosion.

2. *Material for Saccharification*

The material used for hydrolysis, because of its lower cost, is the by-product from various wood-using industries. It consists of mill waste from sawmills and woodworking industries in the form of slabs, edgings, shavings, sawdust, cull veneer and trimmings; of shredded wood waste from the solvent extraction industry for rosin; and of cull wood that may be removed from cut-over wooded areas as a part of a forest management program.

Such material, in addition to having a high moisture content, contains varying amounts of bark, decayed wood and other extraneous materials that yield little or no sugar. The removal of these extraneous substances before hydrolysis would involve expense that could not be justified by the value of the resulting sugar. Therefore it is necessary to hydrolyze the wood with these substances present. In order to determine the amount and effect of these extraneous materials, procedures were developed for the determination of total percentages of potential sugars and of potential fermentable sugars in wood substances. Table IV contains values for the analysis of several wood products.

a. Effect of Bark on Yield of Sugar. The presence of bark was shown to have the following effects on the hydrolysis of wood: (1) the bark with its low percentage of potential sugar and of potential fermentable sugar had a greater effect on the amount of sugar produced than the amount of bark would indicate, because of the retention of sugars in the latter part of the run when decomposition was greater; (2) the yield of sugar was decreased per cubic foot of charge because the actual hydrolyzable material in the digester was decreased; (3) the concentration of the sugar was lower because the amount of sugar produced per unit time was less; (4) more sulfuric acid was required to produce a unit of sugar because of the increase in bulk due to bark; (5) more extraction was required to remove the sugar from the inert residue; (6) extractives from bark increased the nonsugar content of hydrolyzates; (7) the corky nature of

bark decreased the tendency of the residue from hydrolysis to become a hard mass and aided in the removal of the residue; and (8) the bark made it possible to pack more material into the digester without trouble with discharging.

TABLE IV
Potential Sugar Content of Wood Products

Type of material	*Sugar*	
	Total, %	*Fermentable, %*
Douglas fir shavings	67.0	57.0
Douglas fir hogged waste (best material removed)	50.9	42.1
Douglas fir sawdust	63.2	53.4
Douglas fir slabs	56.2	47.4
Douglas fir bark	37.5	26.4
Redwood (bark-free)	52.4	40.3
Redwood mill waste	43.5	35.0
Southern yellow-pine mill waste	59.7	45.5
Southern yellow-pine woods waste	61.8	49.8
Eastern white-pine slabs	60.1	48.3
Southern red-oak shavings	63.6	38.1
Southern red-oak slabs	56.8	42.1
Sugar maple shavings	68.2	48.1
Sugar maple slabs	60.4	42.2
Quaking-aspen woods waste	68.5	50.0
Extracted longleaf pine stumps	58.2	48.0
Extracted ponderosa pine stumps	60.1–62.2	62.6–70.7

b. Effect of Storage on Yield of Sugar. Upon occasion, it is necessary or desirable to store sawdust and other wood waste, or to use wood waste that has been in a pile for some time. Frequently, while in storage, this wood waste undergoes a fermentation that may destroy 10 to 30% of the carbohydrate material in the wood. This fermentation is influenced by the moisture content and temperature of the wood. Old sawdust piles that have undergone fermentation contain only 80 to 85% as much carbohydrate as freshly cut wood.

A similar phenomenon occurs in old dead stumps or timber that has lain on the ground for some time. Fungus growths, such as blue stain, enzymes and other agents convert the hemicellulosic material in wood to water-soluble sugars that are used up by the organism or leached out so that the residue is lower in carbohydrate material. In many cases, a larger portion of the pentosan material is destroyed, and the sugars from such wood products have a higher fermentability.

3. *Procedure for Hydrolysis*

a. Charging the Hydrolyzer. The capacity of a plant for wood hydrolysis is greatly affected by the charge per cubic foot that can be placed in the hydrolyzer. When hydrolysis is carried out in a tall stationary hydrolyzer, it may be assumed that two operations are in progress as the acid passes down the hydrolyzer, namely, the hydrolysis of the carbohydrates and the extraction of the sugar from the hydrolyzed chip.

For the hydrolysis operation, it is desirable that the dilute acid be in contact with all parts of the chip, and that the chip be small enough to permit rapid diffusion of the acid into the chips and of the sugar out of the chips. For this operation, the smaller the wood particle the better, because the diffusion is increased as the smaller size of the chips decreases the distance to be traveled.

In the extraction of the sugars, the flow of liquid down the hydrolyzer is comparable to the action of liquid flowing down the packing of a distilling column. A concentration gradient of sugars develops whereby the concentration increases as the solution passes down the tube. The packing of the charge must be uniform to prevent channeling of the solution. It must be coarse enough to provide a satisfactory rate of flow, and it must be of such a density that the solution comes in contact with as much carbohydrate material as possible. Hydrolysis is usually discontinued as soon as the concentration of sugar being removed from the material reaches a minimum value below which its continuance would be unprofitable. The less the carbohydrate material in the charge in the hydrolyzer, the sooner is this minimum reached, and, therefore, the lower is the percentage of sugar removed from the charge. It is also necessary to avoid overcharging the hydrolyzer, because too dense a charge delays the removal of the sugar until tarry decomposition products have time to form that cement the residual lignin together and delay discharging of the lignin at the end of the run.

When Douglas fir mill waste is used with about 25% by volume of bark, satisfactory operation and discharge of the residue is obtained with charges of 16 to 18 lb. (dry basis) per cubic foot placed in the hydrolyzer. This charging rate is obtained by filling the hydrolyzer loosely with chips, placing the cover on the hydrolyzer, and, with a vent in the bottom open, applying steam rapidly on the top of the charge. The hydrolyzer is again filled and the charge packed with steam. The filling and packing is continued until the desired charge has been introduced. With hogged fuel or sawdust two or three light packings are sufficient. Shavings, which are much more bulky, require more filling and packing. Mixtures

of shavings with other material give normal charging. Hardwood that has a higher density than Douglas fir may be charged in amounts as high as 22 lb. per cubic foot. Lightweight woods, such as redwood, may not be charged in quantities of more than 13 lb. per cubic foot. Filling and packing are accomplished more easily in a hot hydrolyzer.

b. Heating the Charge. The wood charge must be preheated to the temperature of the acid that is to be introduced. If the hydrolyzer is hot, as at the end of a hydrolysis, it is necessary only to heat the wood for the condensate produced by heating to be at a minimum temperature. Heating is accomplished in two steps. The first step is to open a vent to allow air in the charge to be removed. The second is to close the vent when steam flows from it and to introduce the steam to bring the charge to the proper temperature. If the chips are dry, this temperature may be reached in a short time; if wet, longer time and more steam are required. The moisture content of a charge is increased 30 to 50% by heating with steam.

c. Ratio of Dilute Acid to Wood. Acid must be introduced in such concentration and at such temperatures as to bring about the degree of hydrolysis in the time desired and in such quantities as to provide extraction of the sugars in suitable concentration without decomposition or undue dilution. Experience has shown that solutions less acidic than pH 1.7 do not promote satisfactory hydrolysis.

The original charge of dilute acid must be of higher concentration than that required for the normal portion of the run because of the moisture content of the chips. When the starting temperature is 150°C., acid should be introduced in the initial charge to bring the total water content of the chips to a 0.5% acid concentration as soon as possible. For example, if the wood contains 50% moisture, a 480-lb. (dry basis) charge of wood will have associated with it 480 lb. of water. This amount will be increased to about 600 lb. by heating to 150°C. An initial charge of 200 lb. of dilute acid should contain 4.0 lb. of acid to produce the required concentration. Following this initial charge, dilute acid (0.5 to 0.6%) is introduced to provide water for hydrolysis and extraction.

Small amounts of dilute acid gave higher concentrations of sugar but also made a poorer extraction and usually resulted in more decomposition. In most cases when continuous introduction of acid and continuous removal of sugar were used, a satisfactory ratio was 1 part of wood to 2.5 to 3 parts of dilute acid, including the water in the chips due to moisture content and condensed moisture from heating the wood, plus that added with the acid. To produce this ratio, a charge of 400 to 600 lb. of 0.5 to 0.6% acid in addition to the original charge was used.

This procedure differs from that of Scholler,[78] who made no effort to maintain constant acid conditions. Also, because of his using batch addition and batch removal of sugar, it was necessary to have better extraction with each addition. It was necessary, therefore, to use much more water and, as a consequence, more dilute solutions resulted.

If starting temperatures lower than 150°C. are used, the composite acid concentration should be higher than 0.5%; if temperatures 175° to 200°C. are used, the concentration may be less.

After an initial period of about thirty minutes, which is required for the acid to diffuse into the chips, the hemicelluloses are hydrolyzed to simple sugars and no further hydrolysis is required.

Hydrolysis of the stable or alpha cellulose of wood is very slow at 150°C. The calculated half-life with 0.5% sulfuric acid is about 600 minutes. It is, therefore, desirable to raise the temperature as rapidly as conditions permit. If the temperature is raised too rapidly, excessive decomposition of the sugars from the hemicellulose results. In pilot plant operations, the temperature could be raised at the rate of 0.5°C. per minute when the pumping rate was 20 lb. of 0.5% sulfuric acid per minute for a charge of 480 lb. of dry wood. The maximum temperature used was 185°C. All increase in temperature was produced by heating the incoming dilute acid. This differed from the procedure of Scholler, who believed that batch operation and steaming between batches was necessary, and also differed from his operation with the digesters full of liquid. The continuous introduction of acid could be carried out with much lower demand of steam. After about three hours from the time the first acid was introduced the sugar that was being removed fell below 1% concentration and the introduction of acid could be discontinued.

d. Sugar Removal. After the initial period required for diffusion of the acid into the chips and for hydrolysis of the hemicellulose, a valve is opened that permits sugar solutions to be removed continuously at the rate of 20 to 22 pounds per minute. The sugar solutions are removed as rapidly as they flow through the chips, and therefore there is no free liquor in the digester at any time. These solutions flow to a flash tank where they give up volatile products such as methanol, furfural and steam, which may be used for heating the water for hydrolysis. The initial concentration of these sugars ranges from 5 to 15%, depending upon the hemicellulose content of the wood being hydrolyzed. As the flow of sugar continues and the temperature of the hydrolysis is raised, the sugars that are being extracted from the hemicellulose are augmented

(78) H. Scholler, Operating procedures for the Scholler process (1936). Instructions to purchasers of patent rights.

by sugars from the hydrolysis of the stable cellulose. If the rate of increase in temperature is correct, the concentration of sugar will decrease at a uniform rate as the carbohydrate of the charge of wood decreases. If the rate of increase of temperature is slow, there will be a fall in concentration of sugars before the maximum concentration from the stable cellulose is reached. Too rapid an increase in temperature causes the sugar to rise above the initial sugar concentration and excessive decomposition occurs.

Samples of the sugars being removed are cooled to 20°C. and tested for density by a sugar Brix spindle. Analysis of these solutions shows that the reducing sugar content of the solutions is 60 to 70% of the concentration shown by the Brix spindle. If the hydrolysis of hemicellulose is not complete, the ratio of reducing sugar to Brix will be low and will indicate that insufficient acid has been used.

When using similar materials, the color of the solution is an index of the amount of sugar in the solution.

The introduction of acid and removal of sugars are regulated to permit a minimum of dilute acid to flow over the chips in order to maintain as high a concentration as possible. Delay in removing the sugar after it is produced results in decomposition; therefore continuous removal provides the minimum decomposition.

e. Yield of Sugar from Various Wood Products. Table V gives the average yields and concentrations of sugar from various wood products.

f. Recycling of Acid Wood Sugar Solutions. The procedure used in this pilot plant work has involved the use of one hydrolyzer. A number of other procedures are possible.

When the hydrolysis is discontinued at the time the concentration of the sugar being removed falls below 1%, about 10% of the total carbohydrate in the wood remains unhydrolyzed. A portion of this carbohydrate could be recovered by continuing the hydrolysis, but doing so would produce solutions too dilute to mix with the other solutions. These dilute solutions may be used as the hydrolyzing liquor for a fresh batch of chips. Experiments were made in which the initial charge of 2% acid had been added to bring the total acid concentration to 0.5%, after which the dilute acidic sugar solutions from a previous run were introduced. Yields from these experiments indicated that about 6% of the total carbohydrate could be recovered from the residue in this manner.

g. Neutralization. The acidic sugar solutions may be neutralized by any of a number of different substances. Calcium carbonate or lime is preferred because of the low solubility of calcium sulfate. When wood sugar solutions that have been neutralized by lime are used for the production of alcohol, scaling of the alcohol stills due to deposits of cal-

cium sulfate is troublesome because calcium sulfate becomes less soluble as the temperature of the solution is raised. Solutions neutralized and filtered at 100°C. contain 2100 parts calcium sulfate per million; at 121°C.

TABLE V
Hydrolysis of Wood by the Madison Wood Sugar Process[52,76]

Type of wood product	*Hydrolysis time, hrs.*	*Yield of sugar, %*	*Sugar concentration, %*
White-spruce chips	3 1	54.2	5.1
Douglas fir chips	3.0	52.5	5.3
Douglas fir sawdust	3.1	44.7	4.9
Douglas fir hog fuel	3.0	38.7	5.1
Douglas fir bark	2.9	15.3	2.3
Southern yellow-pine woods waste	3.3	50.0	4.8
Southern yellow-pine sawdust	3.1	47.5	4.6
Ponderosa pine chips	3.0	51.5	5.35
Eastern white-pine sawmill slabs	3.1	44.6	4.55
White-fir chips	3.0	53.8	5.40
Western white-pine chips	3.0	46.6	5.0
Sugar pine chips	3.0	46.9	4.0
Western-hemlock chips	3.0	51.5	5.0
Western-larch chips	3.0	54.0	4.9
Western-larch sawmill slabs	3.0	42.0	4.9
Lodgepole pine chips	3 0	51.0	4.9
Spent turpentine chips, longleaf pine stumps	3.1	40.0	4.8
Western red-cedar chips	3.1	46.9	4.3
Redwood chips	3.1	42.6	4.0
Mixed southern-oak shavings	3.0	51.0	5.0
Mixed southern-oak sawdust	3.0	46.9	5.05
Mixed southern-oak sawmill waste	3.1	42.9	4.70
Sugar maple sawmill waste	3.0	48.0	4.75
Yellow-birch sawmill waste	3.1	49.5	5.30
Beech sawmill waste	3.1	46.5	5.04

they contain 1175, and at 140°C. about 530 parts per million. By selecting some elevated temperature, it is possible to remove enough of the calcium sulfate so that scaling does not occur.

The quantity of lime or other base required for neutralization is greater than that required for the sulfuric acid alone because of the

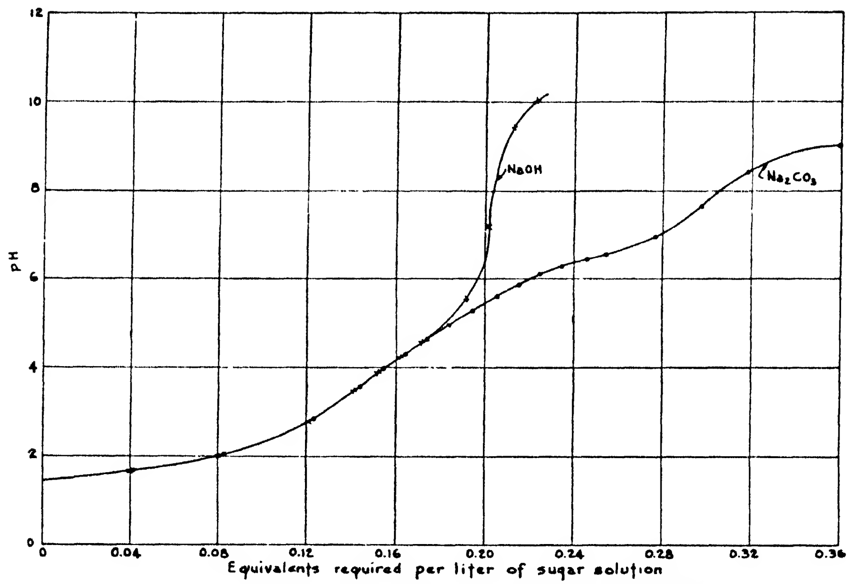

FIG. 3.—Titration curve for acid wood-sugar solution.[75]

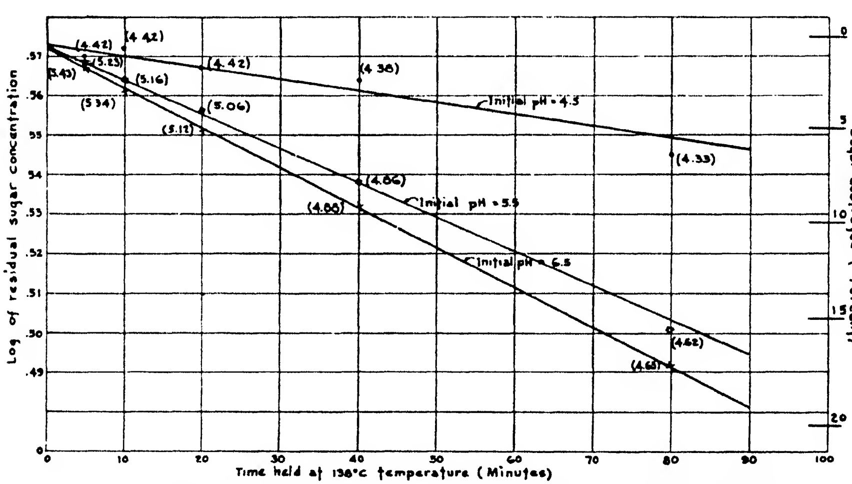

FIG. 4.—Decomposition of wood sugar and change of pH with heating.

presence of acetic and other organic acids. These organic acids have a buffering effect on the neutralization that is illustrated in Fig. 3.

Wood sugars are subject to decomposition if heated for extended periods of time. The rate of this decomposition is increased when the solution is less acid than pH 4.5 (Fig. 4). In all cases the solutions,

where decomposition was great, became more acidic with increasing time. This instability of wood sugars at higher pH values shows that when it is necessary to hold such solutions at elevated temperatures for periods of time, as in the case of evaporation, it is necessary to avoid pH values above 4.5.

4. *By-products of Wood Saccharification*

a. Lignin. The principal by-product of wood saccharification is an insoluble lignin residue. If bark-free wood is hydrolyzed, the yield of lignin residue is 25 to 30% of the weight of the dry wood. Wood waste containing bark gives higher yields of residue because bark is hydrolyzed to a less extent than bark-free wood.

Because the hydrolysis operation is discontinued before cellulose conversion is complete, small amounts of cellulose also remain in the lignin residue.

This lignin residue is characterized by its insolubility in the usual organic solvents. The lignin becomes soluble on heating in alkali or certain organic substances.

On carbonization, this lignin gives yields of about 60% of its dry weight of char, plus tars, acetic acid and methanol.

Because of its high carbon content (65%), this lignin has a high fuel value[79] (10,500 B.T.U. per pound of dry lignin) and may be used to supply the heat for hydrolysis operations.

Lignin may become, through various chemical treatments such as oxidation, hydrogenation and alkali fusion, the source of a whole series of new organic compounds. Lignin from wood hydrolysis has advantages for chemical conversion over lignin from pulping liquors in that it does not require separation from large quantities of water.

b. Methanol. A portion of the methoxyl groups of wood are contained in the hemicellulose portion of wood in the form of esters and acetals. These esters and acetals are hydrolyzed by the action of sulfuric acid on wood. The quantity of methanol produced in this manner ranges from 0.5% of the wood for a softwood to 1.5% for a hardwood. A large portion of this methanol appears in the flash steam from the hydrolyzer and may be recovered by suitable equipment.

c. Acetic Acid. Acetyl groups of wood are combined with the carbohydrate portion of wood.[80] The acetyl groups are hydrolyzed by the action of acid on wood and appear as acetic acid in the hydrolyzate and in steam from the hydrolyzer. The quantity of acetic acid produced

(79) H. Lüers, *Holz Roh- u. Werkstoff*, **1**, 35–40 (1937); *Chem. Abstracts*, **32**, 349 (1938).

(80) W. B. Van Beckum and G. J. Ritter, *Paper Trade J.*, 108, Feb. 16 (1939).

ranges from 1.5 to 5% of the weight of the wood. A portion of this acetic acid is found in the vapors from the hydrolyzer, but most of it remains in the sugar solution and requires neutralization.

d. Furfural. Furfural is produced when pentoses are decomposed in the presence of acids. The amount produced depends upon the severity of the reaction and upon the efficiency of the removal of the products of hydrolysis from the hydrolyzer. The quantity produced is an index of the decomposition occurring in the hydrolysis and of the quantity of pentosans in the wood. In most pilot plant tests on the hydrolysis of softwoods, the furfural produced was about 0.1% of the weight of the wood. Because of the volatility of furfural from dilute solutions, a large portion of the furfural appears in the flash steam from the hydrolyzers.

VII. Utilization of Wood Sugars

Wood sugar solutions, according to Hägglund,[81] contain glucose, fructose, galactose, mannose and pentoses. Mixed with these sugars are found uronic acids, sugar decomposition products, soluble lignin and other organic matter. Many of the methods of sugar analysis may not be used with wood sugars because abnormally high values are obtained. The volumetric hypoiodite method[82] gave values that were frequently 50% higher than the Munson-Walker method.[83] Sugar values were usually about 70% of the readings given by a sugar Brix spindle. If the sugar solutions were neutralized to pH values greater than 4, they also contained large quantities of soluble calcium salts.

These dilute sugar solutions may be used for a number of fermentations, or they may be evaporated to produce sugar solutions suitable for industrial processes.

1. *Alcoholic Fermentation of Wood Sugars*

Wood saccharification processes were developed in most instances to provide sugar for the production of ethyl alcohol by fermentation with yeast. The sugar solutions obtained in these processes have an advantage in that they are sterile and therefore can be used without contamination from the sugar source. They have the disadvantages of being more dilute than sugar solutions from other sources, of containing inhibiting

(81) E. Hägglund, F. Klingstedt, T. Rosenquist and H. Urban, *Z. physiol. Chem.*, **177**, 248 (1928).

(82) C. A. Browne and F. W. Zerban, "Sugar Analysis"; John Wiley & Sons, Inc., New York, 3rd ed. (1941).

(83) Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists, 5th ed. p. 500 (1940).

substances and of requiring that all nutrients must be added. Because of the large volume of liquor they contain in proportion to the amount of fermentable sugar, it is necessary to develop rapid fermentation procedures for commercial installations in order to avoid high expenditures for fermenters. Many investigators have conducted experiments aimed at overcoming the inhibiting properties of wood sugar solutions.[84–88] Products of wood hydrolysis such as acetic and formic acids,[89] carbohydrate decomposition products,[90] and furfural[91–93] are known to have

TABLE VI

Effects of Large Amounts of Inoculum upon the Production of Alcohol

Volume of yeast, %	*Sugar fermented, %*	*Alcohol from total sugar, %*	*Time, hrs.*
1.6	77	36	14.0
2.5	80	42	8.5
3.1	81	44	6 0
5.0	80	45	4.0

inhibiting effects on fermentation. Leonard and Hajny[94] conducted experiments to produce a more easily fermentable hydrolyzate and a more rapid fermentation. The previously noted increase in the rate of fermentation by using higher concentrations of yeast[95–99] with the aid of stirring[100] was found to be effective in producing more rapid fermentations, as shown in Table VI.

(84) A. Partansky, U. S. Pat. 2,203,360 (1940).
(85) H. Scholler, German Pat. 676,967 (1939) and 704,109 (1941).
(86) H. Lüers, G. Fries, W. Huttinger, E. Mopcke and C. Enders, *Z. Spiritusind.*, **60,** 7 (1937).
(87) N. O. Sjølander, A. F., Langlykke and W. H. Peterson, *Ind. Eng. Chem.*, **30,** 1251 (1938).
(88) S. R. Zubkova, N. B. Kochukova and R. M. Zaty, *Biokhimiya*, **1,** 49 (1936).
(89) H. Katagiri, *Biochem. J.*, **20,** 427 (1944).
(90) M. J. Hunter, G. F. Wright, and H. Hibbert, *Ber.*, **71,** 734 (1938).
(91) H. Liang, *Z. physiol. Chem.*, **244,** 238 (1936).
(92) C. J. Lintner and H. J. von Liebig, *Z. physiol. Chem.*, **72,** 449 (1911).
(93) J. J. Reid and I. L. Baldwin, *J. Bact.*, **27,** 29 (1934).
(94) R. H. Leonard and G. J. Hajny, *Ind. Eng. Chem.*, **37,** 390 (1945).
(95) R. H. Hopkins, *Biochem. J.*, **22,** 1145 (1928).
(96) F. F. Nord and J. Weichherz, *Z. Electrochem.*, **35,** 612 (1929).
(97) O. Rahn, *J. Bact.*, **18,** 207 (1929).
(98) A. Slator, *J. Chem. Soc.*, **89,** 128 (1906).
(99) A. Slator and H. Sand, *J. Chem. Soc.*, **97,** 922 (1912).
(100) H. R. Bilford, R. E. Scalf, W. H. Stark and P. J. Kolachov, *Ind. Eng. Chem.*, **34,** 106 (1942).

TABLE VII

Effects of the Addition of Sulfur Dioxide on Amount of Sugar Fermented[94]

Sulfur dioxide added, percent of total sugar	Sugar fermented in 24 hours, percent
0.00	0.0
.03	59.0
.30	77.0

The use of small amounts of reducing agents such as sulfur dioxide has been found helpful, as shown in Table VII.

The neutralization of the acid sugar solution at elevated temperature or heating for a short time after neutralization caused an improvement in fermentation.

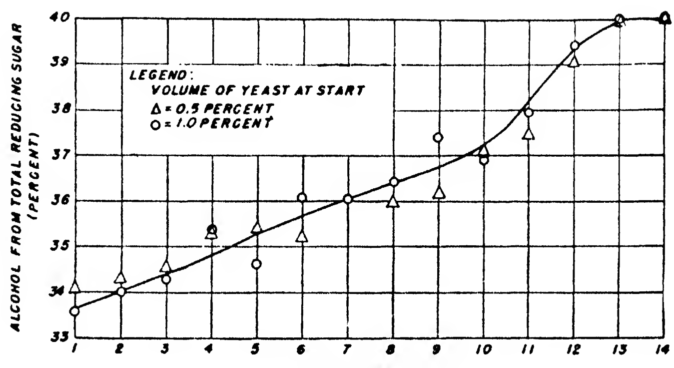

FIG. 5.—Batch alcohol production in the series N batch fermentations of Douglas fir wood hydrolyzate by continuous transfer using 6.3 liters of 5% reducing sugar and 700 ml. of fermented liquor.[101]

Acclimatization of the yeast improved its ability to ferment wood sugar solutions.[101]

Because wood sugar solutions are sterile, they permit yeast to survive under conditions that are suitable for reuse of the yeast. This procedure is similar to that used in the fermentation of sulfite liquor at the Mechanicsville, New York, plant as early as 1913 and in Swedish and German processes since that time. Yeast reuse has also been employed for sulfite waste liquor in Canada.[102]

Recently, yeast reuse has been employed for fermentation of wood sugars. Fig. 5 shows the improvement in alcohol yield when the yeast from a fermented batch is transferred to a new batch of sugar each 24 hours. By using high concentrations of yeast (6 to 12% yeast by

(101) E. E. Harris, G. J. Hajny, M. L. Hannan, and S. C. Rogers, *Ind. Eng. Chem.*, **38**, 896 (1946).

(102) C. A. Sankey and M. M. Rosten, *Pulp and Paper Mag. Canada*, **45**, 171 (1944).

volume) wood sugars can be fermented continuously in four to six hours.[14,103] The yeast is removed from the fermented liquor and mixed with the incoming wood sugar solution before it enters the fermenter.

A strain of *Torula utilis* has been acclimatized by continuous transfer so that it gives high yields by either batch or continuous fermentation in a series of tanks.[104] Fig. 6 shows the improvement in alcohol production in a continuous fermentation with *Torula utilis* No. 3, a strain of yeast obtained from the University of Wisconsin.

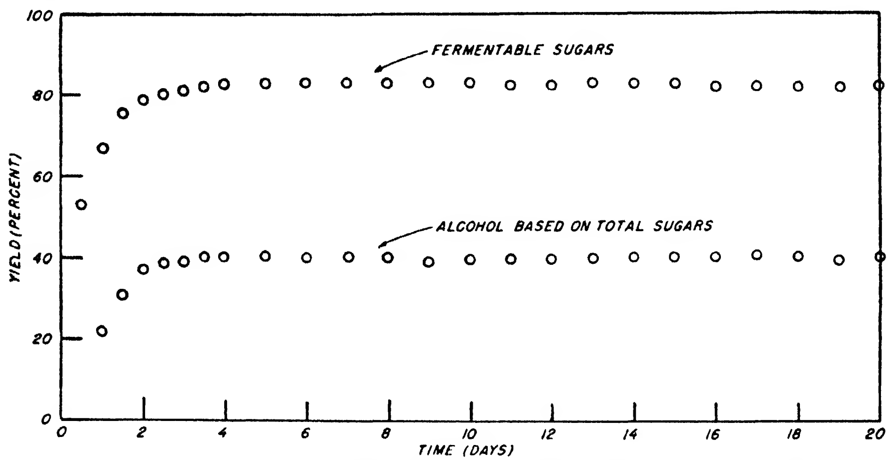

FIG. 6.—Continuous fermentations of wood hydrolyzates by *Torula utilis* in six ten-gallon tanks.[104]

In the work on the fermentation of wood sugars with yeast, no evidence was found of the production of alcohol from pentose sugars. The pentoses from softwoods represent 10 to 12% of the total sugars in solution and constitute a disposal problem in any plant producing alcohol from wood sugars. Nord[105,106] and his associates obtained Douglas fir hydrolyzate containing a high percentage of pentoses, such as were obtained in the forepart of the hydrolysis from the pilot plant operations of the Forest Products Laboratory. These solutions were first subjected to an alcoholic fermentation with yeast. About 60% of the sugars were fermented. The residue was treated with *Fusarium lini* Bolley (FIB) and an additional 1% of alcohol was obtained. This work confirmed that of White and Willaman,[107] who reported that *Fusarium* produced

(103) W. Karsch, German Pat. 692,812.

(104) E. E. Harris, Martha L. Hannan, R. R. Marquardt and Janet L. Bubl, *Ind. Eng. Chem.*, **40,** 1216 (1948).

(105) G. A. Laughman, M. Sookak and F. F. Nord, *Arch. Biochem.*, **6,** 163 (1945).

(106) F. F. Nord and R. P. Mull, *Advances in Enzymol.*, **5,** 165–205 (1945).

(107) Mollie G. White and J. G. Willaman, *Biochem. J.*, **22,** 583 (1928).

alcohol from pentose sugars, and that of Leonard and Hajny,[94] who produced alcohol in 45% yield from wood sugars in seven days. Fermentations with *Fusarium*, however, are very slow, and a considerable amount of developmental work would be required before its use would be considered for an alcohol plant.

2. *Butanol Fermentation of Wood Sugars*

The pentose sugars, as well as the hexoses, are utilized by acetone- and butyl alcohol-producing organisms. Partansky and Henry[108] reported the production of butanol and acetone from wood sugars. This work was continued in the laboratory of W. H. Peterson[87] of the University of Wisconsin. Tables VIII and IX summarize their findings with two organisms.

TABLE VIII
Butyl Alcohol Fermentation of Wood Sugars[87]

Type of material	*Clostridium felsineum*		*Clostridium butylicum*	
	Utilization, %	*Yield of products, %*	*Utilization, %*	*Yield of products, %*
Beech	87	31	90	33.0
Hemlock	90	34	92	34.6
Maple	89	—	—	—
D-Glucose	97	35.6	99	28.7

TABLE IX
Distribution of Products from Butyl-alcohol-producing Organisms[109]

Organism	*Butyl alcohol, %*	*Ethyl alcohol, %*	*Acetone, %*	*Isopropyl alcohol, %*
Clostridium felsineum	57	18	24	—
Clostridium butylicum	60	8	4	25

The application of these organisms decreases the BOD (biological oxygen demand) of such solutions because of the utilization of the pentoses. When sulfite waste liquor is fermented in this manner, the BOD is reduced 34.3%.[109] Scholler reports yields of mixtures of butyl alcohol, acetone, ethyl alcohol and fatty acids equivalent to 13 to 16%

(108) A. M. Partansky and B. S. Henry, *J. Bact.*, **30,** 559 (1935).
(109) A. Wiley, M. Johnson, E. McCoy and W. H. Peterson, *Ind. Eng. Chem.*, **33,** 606 (1941).

of the weight of the wood. Grondal and Berger[110] report similar fermentations of pentoses in sulfite waste liquor after clarification.

3. *Lactic Acid Fermentation of Wood Sugars*

Lactic acid organisms give the same yield of products from pentoses as from glucose when the fermentations are conducted on pure sugars. The residual wood sugars left in the solution after fermentation with yeast and also unfermented wood sugars were fermented[111] with lactic acid organisms. Table X gives the average utilization and yield of products from Douglas fir and a hardwood hydrolyzate.

TABLE X
Lactic Acid Fermentation of Wood Sugar[111]

Type of hydrolyzate	*Sugar utilized, %*	*Sugar recovered as lactic and acetic acids, %*
Douglas fir fermented	75	71.5
Douglas fir unfermented	87	87.0
Hardwood fermented	46	46
Hardwood unfermented	75	70

This fermentation was later applied[112] to sugars from the Bergius process,[113] from the multistage treatment of Sherrard and Davidson,[8] and from the one-stage old American process.[114] In this work 82 to 88% of the total sugars were utilized; the yield of combined acids was 95 to 100% of the sugar; and the acids consisted of 90 to 95% lactic acid and 5 to 10% acetic acid. The time of fermentation for solutions with 7 to 10% reducing sugar was five to seven days. More recently Leonard and Peterson[115] conducted lactic acid fermentations of sugars in sulfite waste liquor and obtained satisfactory utilization of the pentoses.

4. *Production of Butyric and Acetic Acids*

A French process[116] proposes the fermentation of wood sugars for the production of butyric and acetic acids that may be converted into

(110) B. Grondal and H. W. Berger, *Chem. & Met. Eng.*, **52**, 101 (1945).

(111) E. A. Martin, E. C. Sherrard, W. H. Peterson and E. B. Fred, *Ind. Eng. Chem.*, **19**, 1162 (1927).

(112) R. J. Allgeier, W. H. Peterson and E. B. Fred, *Ind. Eng. Chem.*, **21**, 1039 (1929).

(113) F. Bergius, *Chem. Trade J.*, **93**, 356 (1933).

(114) F. W. Kressman, *U. S. Dept. Agr. Bull.* 983 (1922).

(115) R. H. Leonard and W. H. Peterson, *Ind. Eng. Chem.*, **40**, 57 (1948).

(116) C. Berthelot, "Combustibles et Lubrifiants de Remplacement"; Hermann et Cie, Paris (1943); also *Chem. Zentr.*, **1**, 549 (1942).

ketones for use as motor fuel. Wood sugars are neutralized and fermented with *Clostridium pastorianum* and *B. amylobacter*. One organism stimulates the other. The first stage is aerobic and the second anaerobic, according to the following equations:

$$C_6H_{12}O_6 + O_2 + CaCO_3 \rightarrow (CH_3COO)_2Ca + 3\ CO_2 + 2\ H_2 + H_2O$$
$$2\ C_6H_{12}O_6 + CaCO_3 \rightarrow (C_3H_7COO)_2Ca + 5\ CO_2 + 4\ H_2 + H_2O$$

The fermentation required about seven days at 42° to 45°C. at pH 7 to 7.5. In very dilute solutions the yield of acid is about 75% of the weight of the sugar fermented. This type of fermentation appears to be adaptable for a combined operation where ethyl alcohol is produced as a first step and acid as a second from the residual sugars.

When sugar that is produced by the Scholler process is fermented by the acid-producing organism, the yield is only 35% of the total sugar. If the solution is first fermented by yeast, however, a 37% yield of alcohol is produced, and from the residual liquor fermented by the butyric acid organism, a 15.5% yield of acids is produced, making a total recovery of 52.5%.

In order to convert the acids into ketones, they are removed from the fermented liquor and converted into calcium salts. The salts are pyrolyzed at 420°C. to give the ketones.

5. *Production of 2,3-Butylene Glycol from Wood Sugar*

Wood sugar solutions appear to be suitable media for the production of 2,3-butylene glycol, because both hexoses and pentoses are utilized. Perlman[117] carried out fermentations of wood sugar in concentrations up to 17% with *Aerobacter aerogenes* No. 199 (obtained from the Northern Regional Research Laboratory, Peoria, Illinois). The yield of 2,3-butylene glycol and acetoin was about 35% of the fermented sugar. Acclimatization of the *Aerobacter aerogenes* culture to the hydrolyzate makes it possible to ferment higher concentrations of wood sugar.

6. *Yeast Production on Wood Sugars*

The production of yeast protein from wood sugars for use as a food for humans and animals became of national importance in Germany. In 1933 and 1934 the German Ministry of Nutrition made arrangements for yeast production from wood sugar at Tornesch.[118–120] The yield of dry yeast with 50% crude protein was reported to be 25% of the weight of the dry, bark-free wood and the yeast utilized both hexoses and

(117) D. Perlman, *Ind. Eng. Chem.*, **36,** 803 (1944).
(118) H. Scholler, *Zellstoff-Faser*, **32,** 65–74 (May, 1935).
(119) O. Schaal, *Cellulosechemie*, **16,** 7 (1935).
(120) H. Lüers, *Holz Roh- u. Werkstoff*, **1,** 35 (1937).

pentoses. It was found from feeding experiments[121,122] that the yeast was the equivalent of commercial beer yeast. During the war years many improvements were made in the equipment for producing yeast and by the end of 1944 eleven plants[14,123–128] were producing yeast from wood sugars from either wood hydrolyzates or sulfite waste liquor with capacities of more than 22,000 tons of dry yeast per year. Production was about 70% of that value. A report on the utilization of yeast is given by Skoog.[129]

Similar developments were in progress in Sweden[130–132] and in England.[133] The first experiments conducted in America on wood sugar were by Peterson and his coworkers,[134] who made a study of the strains of yeast best adapted for production of the sugars from American species of wood. The strains of yeast used most frequently for food yeast production are the *Torula*, *Candida*, *Hansenula* and *Monilia* types. Of these, the *Torula utilis* is the most important on the basis of the number of plants producing it, although a strain of *Candida arborae* is reported to give satisfactory yields and may, because of its tendency to grow in chains, be more easily processed.

In the growing of food yeast on wood sugar, one of the most difficult problems is that of foaming. This difficulty has been largely overcome by a fermenter designed for use in the production of yeast from sulfite waste liquor in the Waldhof plant at Mannheim. A modification of the fermenter was used for the continuous production of yeast on wood sugars in America.[135] Fermentation periods of 2.5 to 3 hours were obtained with a strain of *Torula utilis*. Yields were 38 to 49% of the total sugar.

(121) F. Honcamp, *Landw. Vers. Sta.*, **121**, 118 (1934).

(122) A. Scheunert and M. Schieblich, *Tierernähr.*, **9**, 173 (1937).

(123) P. L. Pavcek, TIIC Report, Wulf Hefa-fabrik, Dessau, May 29, 1945.

(124) P. L. Pavcek, TIIC Report, Feeding Yeast to Humans, May 30, 1945.

(125) P. L. Pavcek, TIIC Report, Wood Sugar Yeast Manufacture, May 28, 1945.

(126) P. L. Pavcek, TIIC Report, Scholler Process, June 28, 1945.

(127) B. D. David, TIIC Report, Manufacture of Torula Food Yeast from Sulfite Liquor, Zellstoff-fabrik, Waldhof, May 23, 1945.

(128) J. M. Holderby, FIAT Report 619, May 22, 1946.

(129) F. K. Skoog, Food Yeast Production and Utilization in Germany. PB Report 2041 (1945).

(130) S. O. Rosenquist, *Svensk Papperstidn*, **45**, 506 (1942).

(131) S. O. Rosenquist, *Food Ind.*, **16**, 74 (1944).

(132) P. L. Bjørnstad, *Papir-J.*, **29**, 211–12 (1943).

(133) Food Yeast, a Venture in Practical Nutrition; Colonial Food Yeast, Ltd., London (1944).

(134) W. H. Peterson, J. F. Snell and W. C. Frazier, *Ind. Eng. Chem.*, **37**, 30 (1945).

(135) E. E. Harris, J. F. Saeman, R. R. Marquardt, S. C. Rogers and M. L. Hannan, *Ind. Eng. Chem.*, **40**, 1220 (1948).

Pentose sugars in still residues from alcohol production were utilized to the extent of 90% in this equipment.

7. *Biosyn from Wood Sugar*

Another organism that produces protein from carbohydrates is *Oidium lactis*, a fungus. This was described by Peukert[136] as being produced in yields of 60 to more than 100% from various wood sugar solutions. It appears to be able to utilize organic residues other than those that respond to the reducing-sugar test. The product contains 32 to 36% crude protein. Three plants were operating in Germany during the war, producing *Oidium lactis* from wood sugars. These plants had considerable difficulty with contamination and therefore were not able to use a continuous production process as employed for yeast.

8. *Wood Sugars as a Source of Glucose and Sugar for Industrial Processes*

Glucose is used as the raw material in many industrial processes. In some of these, it is used as a thickening material. The concentrated solutions from wood hydrolysis may serve in this capacity.

Glucose is also used as the reducing agent in the preparation of organic compounds. Wood sugars have reducing properties that are equivalent to those of products for like purposes from any source.

Wood sugars may be the source of crystalline D-glucose. The presence of pentoses, however, interferes with the crystallization of the glucose, and therefore only a portion of the glucose may be obtained in crystalline form. Wood sugar molasses may be obtained by the evaporation of the dilute liquors. This operation has now been made possible at low cost because of the development of compression evaporators.[137] This wood sugar molasses may be used as stock feed to supplement the food value of hay and other agricultural products. Provided suitable means can be found to remove extractives and other constituents, these wood sugars could be used as a source of human food.

VIII. Hydrolysis of Wood During Sulfite Process Pulping

The free sulfur dioxide in the sulfite pulping liquor promotes hydrolysis of the more easily hydrolyzable constituents of wood. A wood such as spruce, which contains 70.1% carbohydrate substances, of which 85.3% are potentially fermentable,[67] gives a 50% yield of a pulp containing 97.7% carbohydrate,[138] of which about 80% is fermentable. The

(136) M. E. Peukert, *Cellulosechemie*, **21**, 32 (1943); *Wochbl. Papierfabrik.*, **74**, 77 (1943); German Pat. 744,677 (1943).

(137) A. Latham, *Mech. Eng.*, **68**, 221 (1946).

(138) S. I. Aronovsky and E. C. Dryden, *Paper Ind. and Paper World*, **22**, 253 (1940).

loss in total carbohydrate is about 30%, and the loss in potential fermentable sugars is about 35%. Because the pulping process is designed to delignify the cellulosic residue, rather than to conserve carbohydrate, the digestion is continued for too long a period for the highest yield of fermentable sugar, so that only a fourth of the sugars remains. These sugars, if fermented by yeast, produce about 12.5 gallons of 95% alcohol per ton of wood, or 25 gallons per ton of pulp produced.

IX. Production of Alcohol from Sulfite Waste Liquor

Many plants have been constructed in Europe to utilize the sugars in sulfite liquor for the production of ethyl alcohol or food yeast. Some of these have been in commercial production 35 to 40 years,[139] and have supplied alcohol for motor fuel and protein food for humans and animals.

In Sweden the production of alcohol increased from 8 million liters in 1934 to 23 million in 1936, 32 million in 1939 and 33 million in 1940. Sweden had 33 plants producing alcohol from sulfite waste liquor in 1940. Many improvements have been made with respect to yields and operating costs.

Similar development has occurred in Germany.[140] In 1943 it was reported that 250,000 hectoliters of alcohol were produced from sulfite waste liquor. The preferred method of fermentation was a continuous method in which the yeast was grown on chips or twigs and the neutralized sulfite waste liquor flowed through these chips.[141]

A plant for the fermentation of sulfite waste liquor in America was built at Mechanicsville, New York, by the West Virginia Pulp and Paper Company in 1914.[142] The alcohol stills and some of the other equipment were imported from Germany and were considered the most modern at that time. In comparison to present American stills, these were very inefficient because they were wasteful of steam and did not recover all the alcohol. This plant reused the yeast from a previous fermentation for succeeding fermentations. The plant produced about 221,000 gallons of alcohol per year in 1919.[143] In later years, because of the inefficiency of the alcohol stills with dilute alcohol solutions, molasses was added to the sulfite waste liquor to increase the alcohol content. The plant operated until 1939, when it was closed because of obsolescence.

(139) V. Kinnard, *Foreign Com. Weekly*, **17**, 12 (1944).

(140) *Chem. Ind.*, p. 284 (March, 1943).

(141) George Foth, "Handbuch der Spiritus Fabrikation," Verlag Paul Parey, Berlin (1929).

(142) E. C. Sherrard and F. W. Kressman, *Ind. Eng. Chem.*, **37**, 5 (1945).

(143) E. C. Sherrard and G. W. Blanco, *Paper*, **24**, 15 (July 2, 1919).

Other plants were started at Oregon City, Oregon,[144] and at Appleton, Wisconsin,[145] but developed little further than the pilot plant stage.

With the advent of more efficient stills and more modern yeast-handling equipment, and as the result of the greater demands for alcohol, two modern plants were established, one at Thorold, Ontario, Canada, and another at Bellingham, Washington.

The Thorold plant[102,146] was designed to produce 2000 gallons per day, but improvements in the process have shown that the equipment is capable of producing much more than that amount. The plant uses batch fermentation and reuses yeast according to the Melle process. Claims are made for the production of alcohol at costs as low as 12.4 cents per gallon.

The Bellingham plant was built at the pulp mill of the Puget Sound Pulp and Timber Company as a war project.[147] The cost was about one million dollars, and the plant was built to have a capacity of about two million gallons of alcohol per year.[148] The pulp mill has a capacity of 450 tons of pulp per day, and it is expected that the pulp mill can keep the alcohol plant operating at full capacity.[149,150]

The process consists of removing the sulfur dioxide by stripping, neutralizing the remaining acids by lime and then fermenting by continuous fermentation in a series of tanks.[151–153] This continuous process requires less manpower and permits more economical operation of centrifuges and distillation equipment.

It is claimed that magnesium bisulfite pulping would provide more efficient operation with the possibility of higher yields.[154]

(144) H. B. Tartar, *Paper*, **17**, 14 (March 8, 1916).
(145) Staff Report, *Paper*, **17**, 24 (Feb. 16, 1916).
(146) J. R. Callahan, *Chem. & Met. Eng.*, **50**, 104 (1943).
(147) Staff Report, *Editor & Publisher*, **77**, 58 (Jan. 8, 1944).
(148) Staff Report, *Pulp & Paper Ind.*, **18**, 16 (1944).
(149) Staff Report, *Pulp & Paper Ind.*, **19**, 26 (1945).
(150) H. K. Benson, *Chemurgic Digest*, **5**, 44 (Jan., 1946).
(151) E. O. Ericsson, *Pulp & Paper Ind.*, **19**, 42 (1945).
(152) E. O. Ericsson, *Pulp Mill News*, **69**, 32 (1946).
(153) J. L. McCarthy, *Pulp & Paper Ind.*, **19**, 35 (1945).
(154) G. H. Tomlinson, *Pulp & Paper Mag., Can.*, **45**, 817 (1944).

THE USE OF BORIC ACID FOR THE DETERMINATION OF THE CONFIGURATION OF CARBOHYDRATES

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Contents

I. Introduction

In view of the significance of boric acid for the determination of the configuration of the carbohydrates, it is important first to consider the behavior of boric acid toward polyhydroxy compounds in general, both cyclic and non-cyclic. It had long been known that by adding various compounds to boric acid the acidity was raised,[1] as in the case of glycerol, which was used to determine this weak acid titrimetrically. By determining the change in the conductivity of a considerable number of substances, Magnanini,[2] in Wilhelm Ostwald's laboratory, established this mutual influence more exactly. In the years 1911–1940 these latter investigations were continued and considerably extended in the laboratory at Delft,[3] whereby the simple technique of Kohlrausch and Holborn[4] was almost invariably adopted.

For the polyhydroxy compounds to be examined, the conductivity in water and subsequently (as a rule) in 0.5 *M* boric acid at 25°C. was determined. The increase in this conductivity (called the conductivity

(1) L. Vignon, *Compt. rend.*, **78,** 148 (1874); G. Bouchardat, *ibid.*, **80,** 120 (1875); D. Klein, *ibid.*, **86,** 526 (1878), **99,** 144 (1884).

(2) G. Magnanini, *Gazz. chim. ital.*, **20I,** 428 (1890), **21II,** 134, 215 (1891), *Z. physik. Chem.*, **6,** 58 (1890), **9,** 230 (1892), **11,** 281 (1893).

(3) J. Böeseken, *Ber.*, **46,** 2612 (1913).

(4) F. Kohlrausch and L. Holborn, "Das Leitvermögen der Elektrolyte," B. G. Teubner, Leipzig (1916).

increment, that is, the conductivity observed in 0.5 M boric acid minus the sum of the conductivities of the aqueous solutions of equal concentration of the polyhydroxy compound and of 0.5 M boric acid, expressed Kohlrausch-Holborn units mulitplied by 10^6) is indicated in the following pages as Δ. A detailed description of all these investigations would be beyond the scope of this article; a fairly extensive summary will be found in "Conférence devant la Société chimique de France, Vendredi 16 juin 1933."[5]

For the purpose in view, I shall give a review of the results which are of primary importance for forming a good idea of the action of boric acid on the sugars, in connection with their configuration.

Magnanini[2] had already made it plausible that readily dissociable compounds were formed in aqueous solution, and Van't Hoff[6] had pointed out that polyols with adjacent hydroxyl groups could form cyclic esters, with either five or six atom rings, which would then be stronger acids and, as in the case of mannitol, would have a different rotatory power.

It was found in the first place that aliphatic, non-cyclic glycols without adjacent hydroxyl groups have no effect; they cannot form cyclic complexes. But also most glycols with not more than two adjacent hydroxyl groups are inactive. I therefore assumed that only if two hydroxyl groups are *favorably* situated for the formation of a complex boric acid compound, will it be possible to observe an increase in the conductivity.

In the simple 1,2-glycols the position of the hydroxyl groups is apparently not favorable, and I assumed that this was due to the mutual repulsion of these groups, which impulse they can obey owing to the fact that they can rotate freely around the connecting axis of the carbon atoms to which these hydroxyl groups are bound. This hypothesis has now been proved in several ways.

(1) In the case of compounds with more than two adjacent hydroxyl groups it can be expected that with the mutual repulsion of these groups it will no longer be possible for two of the groups to be 180° apart, as may be the case with the simple glycols, because a third adjacent hydroxyl group prevents this by its repulsion. With an increase in this number of adjacent groups two hydroxyl groups will be more and more favorably situated with reference to one another, as is unmistakably shown by the following results of our determinations (Table I).

(2) If two adjacent hydroxyl groups cannot obey their mutual

(5) J. Böeseken, *Bull. soc. chim.*, [4] **53**, 1332 (1933), *cf. Rec. trav. chim.*, **40**, 553 (1921).

(6) J. H. Van't Hoff, "Die Lagerung der Atome im Raume," F. Vieweg, Braunschweig, 3rd ed. (1908).

repulsion impulse, they will be kept in a certain position of equilibrium. If this position is favorable to the formation of a cyclic complex, a great increase of the conductivity is to be expected; if it is unfavorable, no increase will occur. These consequences have been tested in the three following ways (2a, 2b, 2c).

Table I
Enhancement of the Conductivity of Boric Acid Solutions by Polyols

Substance	*Conc.*	Δ
Glycol	0.5 *M*	0
Glycerol	" "	+ 9
Erythritol	" "	+ 72
Adonitol	" "	+ 90
Arabitol	" "	+357
Xylitol	" "	+625
Mannitol	" "	+685
Dulcitol	" "	+717
Sorbitol	" "	+794

(2a) In the aromatic hydroxyl compounds these groups are held in the plane of the benzene ring, and the formation of a complex is to be expected in the case of the *o*-dihydroxybenzenes and not in that of the *m*- and *p*-dihydroxybenzenes. Our investigation showed that only the ortho derivatives caused a considerable increase of the conductivity (Table II).

Table II
Enhancement of the Conductivity of Boric Acid Solutions by Phenols

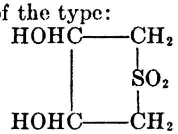
Substance	*Conc.*	Δ
1,2-Dihydroxybenzene (catechol)	1 *M*	681
1,2,3-Trihydroxybenzene (pyrogallol)	1 *M*	909
1,2,4-Trihydroxybenzene (hydroxyhydroquinone)	0.5 *M*	322
1,2-Dihydroxynaphthalene	$\frac{1}{32}$ *M*	65
2,3-Dihydroxynaphthalene	$\frac{1}{200}$ *M*	92
3,4-Dihydroxybenzoic (protocatechuic) acid	$\frac{1}{32}$ *M*	68.7
3,4,5-Trihydroxybenzoic (gallic) acid	$\frac{1}{32}$ *M*	41.1
1,3-Dihydroxybenzene (resorcinol)	0.5 *M*	*neg.*
1,4-Dihydroxybenzene (hydroquinone)	0.5 *M*	*neg.*
1,3,5-Trihydroxybenzene (phloroglucinol)	0.5 *M*	*neg.*

(2b) In the case of alicyclic polyols with adjacent hydroxyl groups it was to be expected that the *cis* glycols would have a positive effect, as the hydroxyl groups are held in a favorable position; on the other hand, the *trans* diols should not show an increase in conductivity because the

hydroxyl groups are fixed in an unfavorable position. This consequence applies when the atoms of the ring are situated in, or approximately in, one plane. Table III shows that this is actually the case with the five-membered ring systems, irrespective of whether they consist entirely of carbon atoms or also contain other atoms, such as oxygen, nitrogen and sulfur. In the case of the saturated six-membered rings something

TABLE III

Enhancement of the Conductivity of Boric Acid Solutions by Cyclic Glycols

Substance[5]	*Conc.*	Δ
Cyclopentane-1,2-diol (*cis*)	0.5 *M*	149
1-Methylcyclopentane-1,2-diol (*cis*)	"	114
Cyclopentane-1,2-diol (*trans*).	"	neg.
1-Methylcyclopentane-1,2-diol (*trans*)	"	"
α-Mannitan	"	776
N-Ethyl-*meso*-tartrimide	"	702
N-Methyl-*dextro*-tartrimide	"	neg.
Indane-1,2-diol (*cis*)	1/7 *M*	63
Indane-1,2-diol (*trans*)	"	neg.
Tetramethylenesulfone-2,3-diol (*cis*)	0.5 *M*	494
Tetramethylenesulfone-2,3-diol (*trans*)	"	neg.
2-Methyl-tetramethylenesulfone-2,3-diol (*cis*)	"	1096
2-Methyl-tetramethylenesulfone-2,3-diol (*trans*)	"	neg.
1,4-Dimethyl-tetramethylenesulfone-2,3-diol (*cis*)	"	1458
1,4-Dimethyl-tetramethylenesulfone-2,3-diol (*trans*)	"	neg.
These sulfonediols are of the type: 		

quite different was observed; no positive action on the conductivity of boric acid was observed with the *cis* 1,2-diols, that is, no more than with the *trans* isomers.[7] Consequently, in the *cis* diols the two hydroxyl groups are situated unfavorably, which is only possible if the six carbon atoms are not in one plane. Thus, this negative behavior of the *cis* 1,2-diol furnishes experimental proof of the theory of Sachse-Mohr.[8] In view of the absence of isomers we must imagine these six-membered rings, at any rate in the gaseous or liquid state, as being in continual movement. The movements will have the character of oscillations round a certain equilibrium position. The angles between the valences thereby remain

(7) J. Böeseken and J. v. Giffen, *Rec. trav. chim.*, **39**, 186 (1920).

(8) H. Sachse, *Ber.*, **23**, 1363 (1890), *Z. physik. Chem.*, **10**, 203 (1892); E. Mohr, *J. prakt. Chem.*, **98**, 315 (1918).

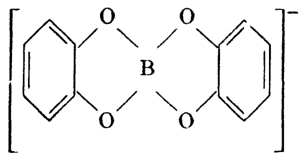
invariably about 109°. It can easily be ascertained that the two adjacent hydroxyl groups may assume a very unfavorable position. As they repel one another, they will tend to assume *trans* relations.[9] With the five-membered rings the angle between the valences of the ring-atoms is 108°, so that they are in one plane; the *cis* 1,2-hydroxyl groups are also practically in one plane and are situated in a favorable position for the binding of boric acid. In the carbohydrates there are frequently six- or five-membered rings with adjacent hydroxyl groups, and we shall see how the hypothesis of the mobility of the six-membered rings as compared with the rigidity of the five-membered rings has clarified the phenomena which make their appearance when the sugars act upon boric acid.

(2c) The α-hydroxy acids cause a very considerable increase in the conductivity of boric acid; this may be explained by assuming that the carboxyl group is hydrated to $C(OH)_3$, as a result of which a very favorable situation prevails in these acids for the formation of a boric acid complex. The non-cyclic β-hydroxy acids, on the other hand, cause no increase of conductivity, evidently because in these compounds, as a result of the repellent action of the hydroxyl and carboxyl groups, they occupy an unfavorable position with reference to one another. Now if these groups are prevented from obeying the repelling action, a positive influence will be observed to occur, if they are kept in a favorable position. The aromatic *o*-hydroxy acids, in which the hydroxyl and carboxyl groups are fixed in the plane of the benzene ring, considerably intensify the acid properties of the boric acid. The *cis*-2-hydroxycyclopentanecarboxylic acids, in which both groups are situated on the same side of the five-membered ring, have a positive action; the *trans* isomers, on the contrary, have no effect. Table IV shows the results of a few of our observations. There is no doubt that these figures are directly connected with the more or less favorable position of the hydroxyl groups which react with the boric acid molecule. Hence, they provide information on the configuration of these compounds. The method is of particular value for distinguishing between *cis* and *trans* isomers of the derivatives of five-membered ring systems. In this manner it was possible for the first time to determine the proper configuration of the two borneolcarboxylic acids.

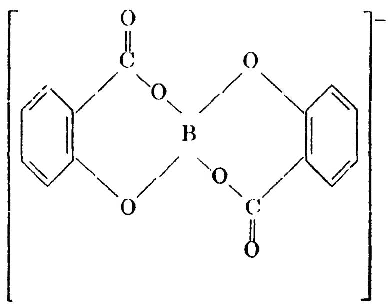
The proof that the formation of certain complexes is involved in the changes of conductivity is furnished by the investigations of Hermans.[10] He established that the compound of catechol, boric acid and potassium

(9) J. Böeseken, *Ber.*, **56**, 2411 (1923).

(10) P. H. Hermans, "Onderzoek naar de ruimtelijke konfiguratie van enkele glycolen"; Thesis, Techn. Boekhandel, J. Waltman, Jr., Delft (1924); see *Z. anorg. allgem. Chem.*, **142**, 83 (1925).

separated by me had the composition:  K^+,

that is to say, that boric acid had fixed two molecules of catechol, with the formation of a spirane with the boron as central atom. This was confirmed by investigations made by Meulenhoff,[11] who succeeded in splitting the boro-disalicylic acid into optical antipodes by means of alkaloid salts, whereby it was also proved that the two-ring



H^+ (alkaloid) systems of this boro-complex cannot be situated in one plane. Hermans also succeeded in preparing compounds of boric acid with glycols which did not increase the conductivity. However, they contained only one molecule of glycol to one molecule of boric acid, and thus were not spiranes.

As a result of the investigations made by Hermans, and later on particularly by Vermaas,[12] the following conclusions were finally established. In the case of the polyols which greatly increase the conductivity, such as mannitol, mannitan, fructose, α-hydroxy acids, salicylic acid and similar compounds, spiranes which possess strongly acidic properties are to be found in aqueous solution, if the excess of boric acid is not too great. The quantity of these strong acids is directly connected, under otherwise equal conditions, with the position of two of the 1,2 (sometimes 1,3) hydroxyl groups. The less favorable the position, the smaller is this quantity, so that finally, especially with an excess of boric acid, no further increase in conductivity is observed. In such cases the action of boric acid on the polyol ceases with the formation of a compound involving one mole of the polyol per mole of boric acid. These compounds, like boric acid itself, are faintly acidic, because they can give up water and pass into neutral compounds.

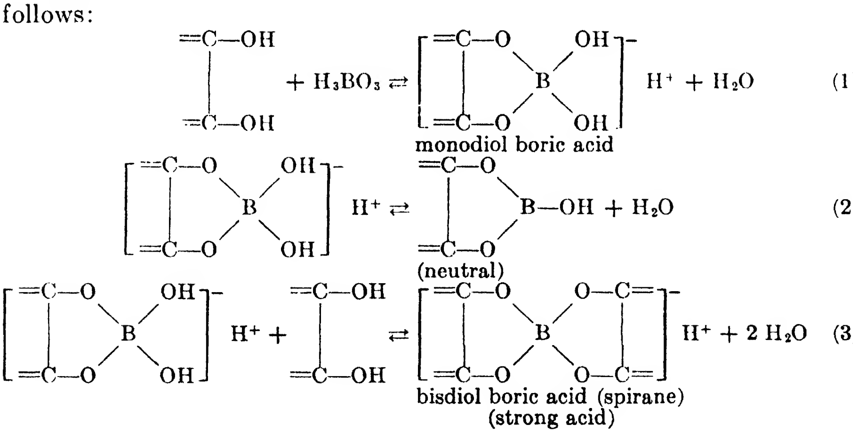
(11) J. Meulenhoff, *Z. anorg. allgem. Chem.*, **142**, 373 (1925).
(12) N. Vermaas, *Rec. trav. chim.*, **51**, 67 (1932).

TABLE IV

Enhancement of the Conductivity of Boric Acid Solution by Hydroxy Acids

Substance	*Conc.*	Δ
$CH_2OH{\cdot}COOH$	0.5 *M*	441
$CH_3CHOH{\cdot}COOH$	0.5 *M*	14213
$(CH_3)_2COH{\cdot}COOH$	0.5 *M*	41307
$C_6H_5CHOH{\cdot}COOH$	0.5 *M*	21500
$(C_6H_5)_2COH{\cdot}COOH$	1/128 *M*	555
$C_6H_{13}CHOH{\cdot}COOH$	1/128 *M*	416
$CH_2OH{\cdot}CHOH{\cdot}COOH$	0.5 *M*	21700
$CH_2OH(CHOH)_4COOH$	0.5 *M*	21636
CH_2OHCH_2COOH	0.5 *M*	neg.
$C_6H_5CHOHCH_2COOH$	0.5 *M*	neg.
$CCl_3CHOHCH_2COOH$	0.5 *M*	neg.
$CH_3CHOHCH_2CH_2COOH$	0.5 *M*	neg.
Salicylic acid	1/64 *M*	1264
2,4-Dihydroxybenzoic acid	1/64 *M*	1501
2,4,5-Trihydroxybenzoic acid	1/64 *M*	1102
2-Hydroxycyclopentanecarboxylic acid (*cis*)	1/8 *M*	96
2-Hydroxycyclopentanecarboxylic acid (*trans*)	1/8 *M*	neg.
5-Methyl-2-hydroxycyclopentanecarboxylic acid (*cis*)	1/8 *M*	89
5-Methyl-2-hydroxycyclopentanecarboxylic acid (*trans*)	1/8 *M*	neg.
Borneolcarboxylic acid (*cis*)	1/64 *M*	530
Borneolcarboxylic acid (*trans*)	1/64 *M*	neg.

The interaction between polyols and boric acid can be formulated as follows:



(1) monodiol boric acid

(2) (neutral)

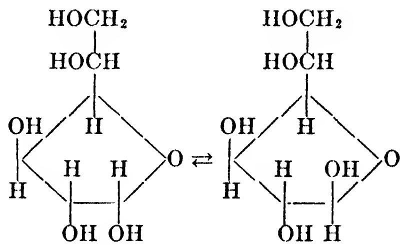
(3) bisdiol boric acid (spirane) (strong acid)

Equilibrium 1 is situated very much to the right; 3 is dependent upon the position of the two hydroxyl groups in the diol. Seeing that the

bisdiol acid, at the dilution involved in this case, is entirely split into ions, the hydrogen ion concentration, under otherwise equal conditions, is a criterion of the more or less favorable position of the hydroxyl groups for the attachment of boric acid to form the spirane.[13]

II. Interaction between the D-Glucoses and Boric Acid

In 1911, when our first investigations of the action of α- and β-D-glucose on boric acid were made,[3,14] the furanose structure was assumed for the monosaccharides having four and more carbon atoms, so that the establishment of an equilibrium between α- and β-D-glucose was represented by the following formulas, written now according to present conventions.



I II

Thus, we could expect that the D-glucose with the configuration I would cause an enhancement in the conductivity of boric acid, whereas that with configuration II would not. Also, that if the mutarotation could be represented by the above formulas, the enhancement caused by compound I would gradually decrease, and the rate of such decrease would be equal to the mutarotation rate under comparable conditions. On the other hand, in the case of the D-glucose with configuration II there would be a gradual increase of conductivity and the rate would be equal to the mutarotation rate. As these consequences were actually confirmed and the common or α-D-glucose showed a diminishing Δ and the β-D-glucose an increasing Δ, the *cis* configuration (I) was attributed to the α-D-glucose, and the *trans* configuration (II) to the β-D-isomer. Table V gives a review of our measurements with the α-D-glucose, whereby $k + k' = \frac{1}{t}\log\frac{r_0 - r_\infty}{r_t - r_\infty}$ and $K + K' = \frac{1}{t}\log\frac{\Delta_0 - \Delta_\infty}{\Delta_t - \Delta_\infty}$ represent the constants of the reversible reaction of the mutarotation and of the changes of the increase in conductivity of a 0.5 M α-D-glucose solution in 0.5 M boric acid at 25°C., respectively. The readings commenced about

(13) J. Böeseken, *Verslag. Ned. Akad. Wetensch.*, **50**, 9 (1944).

(14) (*a*) J. Böeseken and A. v. Rossem, *Rec. trav. chim.*, **30**, 392 (1911); (*b*) J. Böeseken, *Ber.*, **46**, 2612 (1913).

TABLE V

Change of Rotation and Conductivity with Time for Solutions of α-D-Glucose and Boric Acid

(Data of Böeseken and Couvert, Reference 15)

Time, min.	$[\alpha]_D$	$k + k'$	*Time, min.*	*Conductivity*	$K + K'$
0	+95.91°	—	0	94.1	—
5	90.11	0.01129	5	92.5	0.00838
10	85.87	0.01028	10	90.5	0.01007
15	81.42	0.01052	15	89.3	0.00934
20	77.33	0.01076	20	88.3	0.00881
30	70.82	0.01085	30	86.0	0.00907
40	65.52	0.01106	40	84.4	0.00875
60	59.26	0.01066	60	81.9	0.00875
∞	48.36	—	∞	76.7	—
		Average: 0.0108			Average: 0.00904

TABLE VI

Change of Conductivity with Time for Solutions of β-D-Glucose and Boric Acid

(Data of Böeseken, Reference 14*b*)

Time, min.	Δ	$K + K'$
20	111	?
40	116	0.0131
60	118	0.0110
90	121	0.0149
∞	132	—
		Average: 0.0130

four minutes after the D-glucose had dissolved in the boric acid.[15] The readings for the β-D-glucose are shown in Table VI from the measurements in our earliest article.[14b] An irregularity is at once apparent, which we attributed at the time to a contaminant of the β-D-glucose. Later observations[16] showed that this irregularity actually existed and that $(K + K')$ decreased progressively during the mutarotation; evidently the transition β → α-D-glucqse could not be solely a change in position of the 1-hydroxyl group with reference to the plane of the ring. However, the observations with α-D-glucose and the consequences deduced therefrom appeared to be confirmed by the behavior of the methyl α- and β-D-glucosides and of the corresponding methyl tetra-

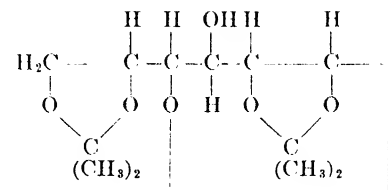
(15) J. Böeseken and H. Couvert, *Rec. trav. chim.*, **40,** 354 (1921).

(16) R. Verschuur, *Rec. trav. chim.*, **47,** 123, 423 (1928); Thesis, Delft (1927).

methyl-D-glucosides, none of which showed an increase of the conductivity.[15] The former lacked the C1 hydroxyl group, the latter the C1 and C2 hydroxyl groups; thus none of them has adjacent hydroxyl groups in a *cis* relation in the ring.

The negative behavior of sucrose also confirmed our view, for all the eight hydroxyl groups are unfavorably situated and no increase in the conductivity was to be expected.

The comparatively easy formation of 1,2:5,6-diisopropylidene-D-glucofuranose[17] appeared to support this deduction. But the failure



to prepare the 5,6-isopropylidene derivatives of ordinary methyl α- and β-D-glucosides[18] was in itself an indication that there cannot be any adjacent hydroxyl groups in positions 5 and 6.

Fischer succeeded, it is true, in preparing a 5,6-isopropylidene compound from the "γ-methyl glucoside," but it was found subsequently that this glucoside is a derivative of D-glucofuranose. A serious objection to a furanose configuration for crystalline α-D-glucose is the *amount* of the increase of the conductivity. If we compare it with the increase expected of a five-membered ring with adjacent hydroxyl groups in a *cis* relation, especially in heterocyclic compounds, it will be found to be much smaller (Table III). Other aldoses, with the exception of D-mannose, show similar small increases in conductivity.

The investigations of Haworth and coworkers have proved that the normal methyl glycosides are pyranosides and thus possess six-membered rings. The sugars themselves are very closely related to the normal glycosides and are generally assumed to have the pyranose structure. Such structures apply to the crystalline sugars, but are not quite correct in the case of aqueous solutions. If α-D-glucose in aqueous solution consisted solely of α-D-glucopyranose, it would hardly be capable of exercising any influence on the conductivity of boric acid, presumably no more than does inositol.[19] It is certain that when the D-glucopyranose is dissolved, a substance is formed with a greater influence than a pyranose

(17) E. Fischer, *Ber.*, **28**, 1165 (1895).
(18) J. Böeseken, *Rec. trav. chim.*, **40**, 356 (1921).
(19) J. Böeseken and Anny Julius, *Rec. trav. chim.*, **45**, 489 (1926).

could create. This may be the furanose, but it may also be a hydrate of the pyranose or the open-chain sugar (see below). If it were solely the furanose, no increase in conductivity could occur at first when β-D-glucose was dissolved, as neither the β-D-glucopyranose nor the β-D-glucofuranose can exercise a positive influence on the conductivity of boric acid. The increase in the conductivity of β-D-glucose can be explained as follows.[16] If we regard the change as a reversible process, then $K + K' = \frac{1}{t} \log \frac{\Delta_0 - \Delta_\infty}{\Delta_t - \Delta_\infty}$. The fact that this "constant" *decreased* from 0.0230 to 0.0076 in the later experiments of Verschuur,[61] indicates that substances are first formed which greatly influence the conductivity. As already remarked, β-D-glucofuranose cannot be among these substances. α-D-Glucofuranose is also out of the question, for if it were formed the change in the increase in conductivity would keep pace with the mutarotation, which is not the case in the first stage.

When α-D-glucopyranose is dissolved these anomalies are not observed,[16] for $K + K' = 0.0109$, a value practically equal to the mutarotation constant, 0.0107. In this case the influence of the intermediate product is overshadowed by the presence of α-D-glucofuranose, which exercises a very great influence on the conductivity because it is a five-membered ring with two adjacent *cis* hydroxyl groups. The mutarotation process may now be symbolized as follows:

$$\begin{array}{ccccc}
\alpha\text{-D-Glucopyranose} & \rightleftarrows & \text{Intermediate product} & \rightleftarrows & \alpha\text{-D-Glucofuranose} \\
\upharpoonleft\downharpoonright & & & & \downharpoonleft\upharpoonright \\
\beta\text{-D-Glucopyranose} & \leftrightarrows & \text{Intermediate product} & \leftrightarrows & \beta\text{-D-Glucofuranose}
\end{array}$$

These intermediate products are probably D-glucopyranoses hydrates, whose rotatory power may be assumed to be equal to that of the non-hydrated sugars. It is improbable that these hydrates have an open chain; the writer assumes that the shifting from α-pyranose to α-furanose will take place without opening of the ring, the hydroxyl group remaining undisturbed, so that this intramolecular transformation will have little influence on the rotatory power. The formation of the open-chain sugar is not precluded, but its concentration must be very low.

From this point of view, both the pyranoses and the furanoses may participate in the mutarotation reaction, and both α- and β-D-glucopyranose will have similar mutarotation constants. We may expect a heterocyclic five-membered ring with two adjacent *cis* hydroxyl groups to have a Δ of about 1000 units for 0.5 *M* solutions. In the case of D-glucose it is between 93 and 80; hence, the concentration of α-D-glucofuranose is probably less than 10 per cent. This quantity is sufficient, however, to explain the formation of derivatives of the furanoses.

III. The Effect of D-Galactose on the Conductivity of Boric Acid

In our first measurements[20] with solutions of 0.5 M α-D-galactose in 0.5 M boric acid, the constants of the mutarotation and of the change in the conductivity agreed very well.

$$k + k' \text{ (rot.)} = \frac{1}{t} \log \frac{r_0 - r_\infty}{r_t - r_\infty} = 0.0145$$

and

$$K + K' (\lambda) = \frac{1}{t} \log \frac{\Delta_0 - \Delta_\infty}{\Delta_t - \Delta_\infty} = 0.015$$

Since both values decreased and since the furanose structure was attributed to this sugar, the *cis* configuration was given to the common or α-D-galactose and the *trans* configuration to the β-D-isomer. A new investigation[16] showed that both the rotation and the conductivity of the α-D-galactose were less and those of the β-D-galactose greater at equilibrium than initially, but, particularly in the first minutes after the dissolution, considerable deviations occurred. In the first eleven minutes after the dissolution of the α-D-galactose the conductivity *rose*. After thirteen minutes a steady decline set in and the reaction constant (0.0128) was appreciably smaller than the mutarotation constant (0.015). The mutarotation constant of the β-D-galactose was 0.0134; no true reaction constant was found for the change (increase) in the conductivity, as was previously the case with β-D-glucose. The $K + K'$ value *decreased* from 0.0420 to 0.024. These irregularities indicate that both with α- and with β-D-galactose, substances formed in the first stage after the dissolution have a greater influence on the conductivity than can be attributed to the two known sugars and that in part these substances rapidly disappear. Verschuur[16] prepared the crystalline equilibrium mixture with $[\alpha]^{20}_{D}$ $+81.1°$, which remained constant upon dissolution. The conductivity was indeed found to increase considerably in the first twenty-six minutes from 61.2 to 64.9, while the "constant" decreased from 0.0171 to 0.0031. Now, it is certain that a six-membered ring is present in the crystalline sugars, and it is hardly to be expected that this type of ring will cause an increase of the conductivity in boric acid; hence, substances must be formed of which two of the adjacent hydroxyl groups are favorably situated. This effect can be partly ascribed to α-D-galactofuranose. There must, however, be other forms present during the first few minutes, for in the case of the β-D-galactose a substance is formed which exhibits a higher Δ; it disappears rather quickly and cannot be the β-D-galactofuranose, because this isomer could not be expected to give a positive Δ, any more than could the β-D-glucofuranose. This part can probably be

(20) J. Böeseken, *Proc. Roy. Neth. Acad. Sci.*, **24**, 1624 (1916).

played by a hydrate of the sugar. The whole process could then be symbolized as follows:

$$\begin{array}{ccccc} \alpha\text{-D-galactopyranose} & \rightleftharpoons & \text{preliminary substance} & \rightleftharpoons & \alpha\text{-D-galactofuranose} \\ \updownarrow & & & & \updownarrow \\ \beta\text{-D-galactopyranose} & \rightleftharpoons & \text{preliminary substance} & \rightleftharpoons & \beta\text{-D-galactofuranose} \end{array}$$

As the conductivity of the equilibrium mixture in 0.5 *M* boric acid amounts to about 65 units, the quantities of D-galactofuranoses are smaller than for D-glucose solutions, but on the other hand the mutarotation proceeds somewhat more rapidly. It may also be observed that in pure water both isomers also show a gradual increase in conductivity, which however is much less than in 0.5 *M* boric acid.

The investigations by Riiber and coworkers[21] of the changes in volume and refraction of sugar solutions during the mutarotation almost coincided with these observations. With D-glucose they found a dilatation which paralleled the mutarotation; with D-galactose at first there was a dilatation which was followed by a contraction. Verschuur[16] pointed out the analogy between these phenomena and his observations and also proved that the rotation of the α-D-galactose in the first six minutes decreased to a much greater extent than in the subsequent course of the mutarotation.[22] These investigations thus proved that the D-galactopyranoses in aqueous solutions are partially converted to other substances. The boric acid examination enables us to decide, first, that these are substances in which two hydroxyl groups are more favorably situated for the formation of a boric acid complex than is the case for the D-galactopyranoses, and second, that a cyclic hydrate of the sugars is probably formed initially; subsequently a transformation to the furanoses occurs and in the second phase the α-D-galactofuranose is largely responsible for the increase in conductivity.

IV. The Action of β-D-Mannose on Boric Acid[15]

Measurements of the influence of boric acid on the rotation and conductivity of β-D-mannose yielded the results shown in Table VII. For β-D-mannose both the rotation and the increase in conductivity become greater and these phenomena run practically parallel. Thus, it is not surprising that we assumed at the time that β-D-mannose should be allotted the *trans*-mannofuranose configuration, and its isomer, which

(21) C. N. Riiber, *Ber.*, **56**, 2185 (1923); C. N. Riiber and J. Minsaas, *ibid.*, **59**, 2266 (1926); C. N. Riiber, J. Minsaas and R. T. Lyche, *J. Chem. Soc.*, 2173 (1929).

(22) See also G. F. Smith and T. M. Lowry, *J. Chem. Soc.*, 666 (1928), who on the strength of these deviations assumed a third modification of the D-galactose, and see also H. S. Isbell and W. W. Pigman, *J. Research Natl. Bur. Standards*, **18**, 141 (1937).

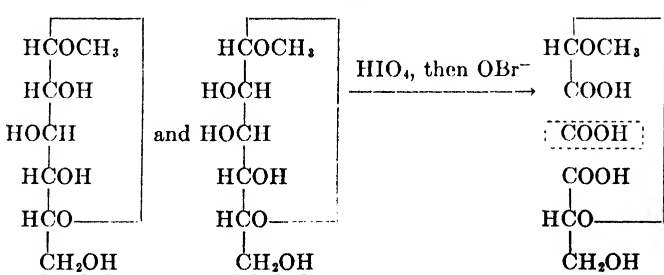
was not known then, the *cis* configuration. Although this original conclusion was incorrect, we do know that when β-D-mannose is dissolved, the hydroxyl groups of the substances present are not so favorably situated at the beginning as they are when equilibrium is reached. The investigations of Haworth and coworkers have demonstrated that the methyl D-mannosides are pyranosides. In all likelihood, the D-manno-

TABLE VII

Change of Rotation and Conductivity of β-D-Mannose in Boric Acid Solutions

Time, min.	$[\alpha]_D$ (0.5 *M* β-D-*mannose* + 0.5 *M* H_3BO_3)	$k + k' = \frac{1}{t}\log\frac{r_0 - r_\infty}{r_t - r_\infty}$	*Variations of the conductivity*	$K + K' = \frac{1}{t}\log\frac{\Delta_0 - \Delta_\infty}{\Delta_t - \Delta_\infty}$
0	− 9.34°	—	314.3	—
5	− 2.50	0.02970	323.1	0.03004
10	+ 2.83	0.03144	329.2	0.02967
15	+ 5.39	0.02829	333.8	0.03012
20	+ 8.72	0.03141	338.3	0.03462
30	+11.22	0.02959	—	—
50	+13.33	0.02791	442.5	0 02949
∞	+14.28	—	344.4	—
	Average: 0.0297		Average: 0.03079	

pyranose structure may also be assigned to the crystalline forms of the sugar. Moreover, Jackson and Hudson[23] proved that methyl α-D-mannopyranoside has a *trans* configuration. Upon oxidation with periodic acid this glycoside yielded the same dibasic acid as that obtained from methyl α-D-glucopyranoside and methyl α-D-galactopyranoside.

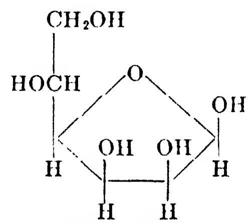


Methyl α-D-glucopyranoside Methyl α-D-mannopyranoside

The β-D-mannose examined by Couvert[15] may then certainly be given the *cis* configuration. Since the conductivity measurements are not

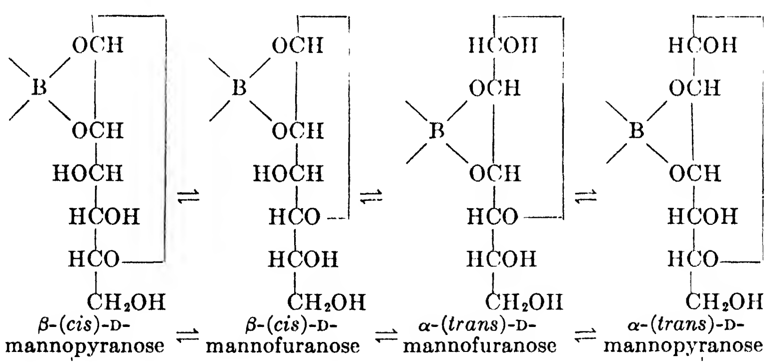
(23) E. L. Jackson and C. S. Hudson, *J. Am. Chem. Soc.*, **59**, 994 (1937).

compatible with a simple change in configuration at the reducing group, a change which would certainly induce a decline in the conductivity increment, other phenomena also must play a part in the solution of β-D-mannose. Substances must be formed during solution which cause a great increase in conductivity and when equilibrium is reached these substances must be present in rather large quantities. They will very probably be D-mannofuranoses, for *cis*-D-mannofuranose has three very favorably situated adjacent hydroxyl groups; if these furanoses are formed from *cis*-D-mannopyranose, the conductivity will undoubtedly increase, even if the transfer of a C1 hydroxyl group, resulting in the



β-D-Mannofuranose

conversion of a *cis*- to a *trans*-configuration, occurs at the same time. What takes place in boric acid solution might be symbolized as follows.



As a matter of fact the high value of Δ in the equilibrium solution (344.4) is a sign that a very large proportion of D-mannofuranoses is present; in this connection it must be borne in mind that the *cis* as well as the *trans* modification has in this case two favorably situated adjacent hydroxyl groups attached to carbon atoms 2 and 3. Since α-(*trans*)-D-mannose has not been examined, no further conclusions can be drawn.

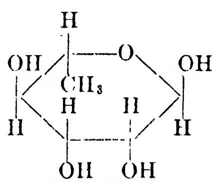
Thus, it is clear that the transition from the *cis* pyranose to the *trans* pyranose determines the increase in rotation as well as the increase in conductivity in 0.5 *M* boric acid, but that the latter is no longer a con-

sequence of a simple change of configuration at carbon 1. This change will cause a decrease in the conductivity, but the simultaneous displacement of the boric acid from the *cis*-1,2-hydroxyl groups of the six-membered ring to the *cis*-2,3-hydroxyl groups of the five-membered ring will result in a very marked increase in the conductivity increment, which will more than compensate for the decrease.

Finally, I would draw attention to the reaction of D-mannose with acetone;[24] the 2,3:5,6-diisopropylidene-D-mannofuranose is very readily formed.

V. Interaction between Boric Acid and α-L-Rhamnose

α-L-Rhamnose is closely related to α-L-mannose; here, too, the increase in conductivity with boric acid rises and the *negative* rotation *declines*.



α-L-Rhamnopyranose

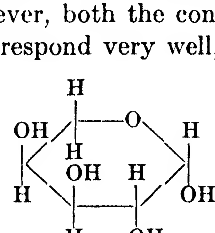
$$k + k' = \frac{1}{t} \log \frac{r_0 - r_\infty}{r_t - r_\infty} = 0.0785$$

$$K + K' = \frac{1}{t} \log \frac{\Delta_0 - \Delta_\infty}{\Delta_t - \Delta_\infty} = 0.0758$$

Since $\Delta = 50.0 \rightarrow 64.7$, only small quantities of furanoses can be present and the transition of the pyranoses is hardly disturbed. Accordingly the common or α-L-rhamnose is the *trans*-L-rhamnopyranose.

VI. Interaction between Boric Acid and β-L-Arabinose

In the case of β-L-arabinose the strongly positive rotation decreases and the conductivity increment likewise decreases. As Δ is rather large (for the equilibrium mixture, 119.7), furanoses or hydrates must be taken into account. However, both the constants of the mutarotation and of the change of Δ correspond very well, namely, $k + k' = 0.0582$,



β-L-Arabinopyranose

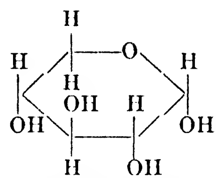
and $K + K' = 0.0561$, so that the above formula may be attributed to the crystalline isomer. Apparently, however, these pyranoses are partly converted into the corresponding furanoses or hydrates. No measure-

(24) K. Freudenberg and R. M. Hixon, *Ber.*, **56**, 2119 (1923); J. C. Irvine and A. F. Skinner, *J. Chem. Soc.*, 1089 (1926).

ments have been made with α-L-arabinose. It may be added that 1,2:3,4-diisopropylidene-L-arabinopyranose is fairly readily formed.[25]

VII. Interaction between Boric Acid and α-D-Xylose

α-D-Xylose exercises a considerable influence on the conductivity of boric acid. Since Δ has the value 210.2, rather large quantities of furanoses or hydrates are formed. This is probably the basis for the discrepancy between the mutarotation constant (0.03425) and the constant of the change of Δ(0.0383). Otherwise the two quantities decrease uniformly, so that the *cis* configuration can be attributed to freshly dissolved α-D-xylose. An unexpected feature is that with acetone α-D-xylose forms a *di*isopropylidene compound.[26]



α-D-Xylopyranose

No measurements were carried out with the β-D-xylose, since it is not known in crystalline form.

VIII. Action of the α- and β-Lactoses and of β-Maltose on Boric Acid

In 1926 it appeared very unusual that neither milk sugar nor maltose exercised a positive (augmentative) influence on the conductivity of boric acid, since both contain a D-glucose radical and therefore a positive influence on the conductivity was to be expected with both sugars, just as with D-glucose. From the nature of the products of hydrolysis of the entirely methylated sugars and of the corresponding bionic acids, it has meanwhile been ascertained that the D-galactose or D-glucose radicals are attached to the 4th carbon atom of a D-glucose radical.[27] Consequently, *they cannot pass into the furanose isomers*, and the formation of a substance with favorably situated adjacent hydroxyl groups at positions 1 and 2 is prevented.

This negative behavior proves, moreover, that during the mutarota-

(25) E. Fischer, *Ber.*, **28**, 1163 (1895); H. Ohle and Gertrud Berend, *Ber.*, **60**, 810 (1927).

(26) O. Svanberg and K. Sjöberg, *Ber.*, **56**, 863 (1923); K. Freudenberg and O. Svanberg, *ibid.*, **55**, 3239 (1922).

(27) W. N. Haworth and S. Peat, *J. Chem. Soc.*, 3094 (1926); W. N. Haworth and C. W. Long, *ibid.*, 544 (1927).

tion only traces of the open-chain sugar can be formed and directs attention to the great part which the furanoses play in solutions of aldonic monosaccharides. Our hypothesis as to the presence of these five-membered ring sugars in these monosaccharides is supported by this negative behavior. Although no increase in conductivity increment was observed, a remarkable difference between the α- and β-lactoses as regards their behavior toward boric acid was found.

With α-lactose the *negative* influence was less than with the β-lactose and during the mutarotation reaction the conductivity increased in the case of the former, while it became less in the case of β-lactose. The two isomers were very carefully purified. Table VIII gives a review of the measurements.[28]

TABLE VIII

Conductivity of α- and β-Lactose[28]

Concentration		Conductivity at 25°C in K-Hb × 10^{-6} units						
			β-Lactose			α-Lactose		
Lactose	H_3BO_3	H_3BO_3	Without H_3BO_3	With H_3BO_3 Found	With H_3BO_3 Calcd.[a]	Without H_3BO_3	With H_3BO_3 Found	With H_3BO_3 Calcd.[a]
0.5 M	0.5 M	27.8	12.3	34.8 → 37.5	40.1	12.9	39.1 → 37.9	40.7
0.25 "	0.25 "	11.8	8.3	20.6 → 22.2	20.1	8.7	23.0 → 22.1	20.5
0.125 "	0.125 "	4.8	5.4	10.1 → 10.7	10.2	5.6	11.6 → 11.0	10.4
0.25 "	0.5 "	27.8	8.3	32.9 → 33.7	36.1	8.7	35.1 → 34.3	36.5
0.125 "	0.5 "	27.8	5.4	29.0 → 29.9	33.2	5.6	31.0 → 30.5	33.4

[a] Sum of columns 3 and 4 or 3 and 7.

There is no doubt that for β-lactose the conductivity in boric acid increases and for α-lactose it decreases. Thus, in α-lactose two hydroxyls are situated more favorably than in β-lactose. On the strength of this investigation we may attribute the *cis* configuration to α-lactose and the *trans* to the β-lactose. However, the observations were not accurate enough to serve as a basis for the calculation of a constant for the reversible change of the conductivity; at most we can say that this change is of the same order of magnitude as that of the mutarotation.

Maltose,[29] prepared from starch by treatment with barley malt extract and repeatedly recrystallized from 90% alcohol, showed the same

(28) J. Böeseken, *Rec. trav. chim.*, **61**, 85 (1942); R. Verschuur, Thesis, Delft, p. 85 (1926).

(29) R. Verschuur, Thesis, Delft, pp. 53–60 (1926).

phenomenon as β-lactose. The conductivity of the solutions in boric acid of various concentrations was invariably less than the sum of the Δ of boric acid and of the pure sugar, but it increased until equilibrium was reached. Table IX presents a summary of a large number of observations. This increase of Δ shows that the hydroxyl groups are less

TABLE IX
Conductivity of β-Maltose in Boric Acid Solutions

Concentration		*Cond. of H_3BO_3*	*Conductivity in K-Hb $\times 10^{-6}$ units*		
			Maltose alone	*Maltose with H_3BO_3*	
Maltose	*H_3BO_3*			*Found*	*Calcd.*[a]
1.0 M	0.5 M	27.8	17.4	28.5 → 33.3	45.2
0.5 "	0.5 "	27.8	14.6	33.3 → 36.9	42.4
0.25 "	0.5 "	27.8	9.8	32.8 → 35.1	37.6
0.125 "	0.5 "	27.8	7.1	31.5 → 32.9	34.9
0.250 "	0.250 "	11.8	9.8	17.3 → 18.7	21.6

[a] Sum of columns 3 and 4.

favorably situated in the known form of maltose than in the α-isomer, and that it is therefore the β-(*trans*)-maltose, as indeed is generally assumed. The increase in conductivity, however slight, is regular in this case. In several series of observations Verschuur[29] found for $K + K' = \frac{1}{t} \log \frac{\Delta_0 - \Delta_\infty}{\Delta_t - \Delta_\infty} \times 10^4$ the values 83.2, 84.6, 85.5 and 87.1 (average 85.1); the constant of the mutarotation in water averaged 81.4 and in 0.5 M boric acid, 94.7.

IX. Effect of D-Fructose and L-Sorbose on the Conductivity of Boric Acid

The increase in conductivity is very much greater for ketoses than for aldoses. In the case of a D-fructose solution of 0.5 M concentration in 0.5 M boric acid the conductivity at 25° is 803.7 Kohlrausch-Holborn units;[15] at a sugar concentration of 0.1 M in 0.5 M boric acid it is 208.7 units;[30] and the value is 96.2 units for 0.1 M D-fructose in 0.1 M boric acid.[30] For L-sorbose of 0.1 M concentration in 0.1 M boric acid the conductivity is 230.0 units.[31]

The changes in rotation and in conductivity increment could not be investigated with 0.5 M D-fructose, because in the strongly acidic medium

(30) R. Verschuur, Thesis, Delft, p. 74 (1927).
(31) J. Böeseken and J. L. Leefers, *Rec. trav. chim.*, **54**, 865 (1935).

TABLE X

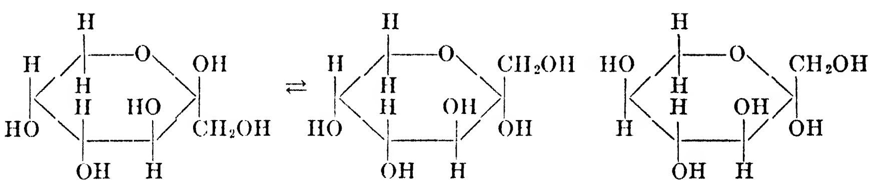
Change of Rotation and Conductivity of D-Fructose in Boric Acid Solutions

1	2	3	4	5	6	7	8
D-*Fructose conc.*	H_3BO_3 *conc.*	*Mutarotation constant* $(k + k')$	*Constant for change of conductivity* $(K + K')$	$[\alpha]_D$ *(equil.)*	*Specific conductivity (equil.)*	*Observed change in* $[\alpha]_D$	*Observed increase in specific conductivity*
0.1 *M*	0.1 *M*	0.156 & 0.164	0.168 & 0.171	−86° & −88°	90 6 & 96	−106 → −88°	71.2 → 96.2
0.1 "	0.2 "	0.257	0.24	−88.1	136.0	− 99.7 → −88.1	122 → 136
0.1 "	0.3 "	0.30	0.29	−88.1	157.9	− 95.6 → −88.1	145.7 → 157.9
0.1 "	0.4 "	—	0.37	—	187.2	—	179.0 → 187.2
0.1 "	0.5 "	—	0.43	—	208.7	—	195.5 → 208.7

these changes took place too rapidly. They were investigated by Couvert and Verschuur with 0.1 *M* D-fructose solutions in boric acid solutions of various concentration (Table X). The figures shown in columns 5 and 6 refer to the values after the equilibrium has been reached. The negative specific rotation decreases, and the conductivity increment becomes greater.

It will be observed that the constants correspond rather well and we may conclude that in common fructose the hydroxyl groups occupy a less favorable position than in the isomer (or isomers) formed during the mutarotation reaction.

Investigations by Haworth and many others have made it probable that a D-fructopyranose structure is to be assigned to crystalline D-fructose. Formulas III and IV below illustrate the interconversion of



III IV V

D-Fructopyranose isomers L-Sorbose

D-fructopyranose isomers. When one seeks to allocate to crystalline D-fructose one of these pyranose structures in preference to the other, there arise several uncertainties. First, there is evidence from the work of Isbell and Pigman[32] that the mutarotation of the known form of D-fructose represents mainly a pyranose to furanose conversion. Such a conversion must cause an increase in conductivity as well, because in that case a five-membered ring with two adjacent *cis* hydroxyl groups is formed. Next, carbon atoms 1 and 2 carry hydroxyl groups and carbon atom 1 is not a part of the pyranose or furanose ring. Lastly, it should be borne in mind that the ring atoms do not lie strictly in a plane and *cis* hydroxyl groups may therefore be unfavorably situated for binding boric acid.

One of the differences between ketoses and aldoses is that the ketoses have a CH_2OH group bound to the reducing carbon atom instead of a hydrogen atom. In the absence of further experimental results on the action of boric acid on ketoses it seems best to defer allocations and to emphasize only that ketoses form much greater quantities of the boric acid complex than do aldoses. This is apparently due to the fact that

(32) H. S. Isbell and W. W. Pigman, *J. Research Natl. Bur. Standards,* **20,** 773 (1938).

the accumulation of hydroxyl groups in the vicinity of the reducing carbon atom is greater for ketoses than for aldoses. The fact that the conductivity increment of the equilibrated solution is very much greater under comparable conditions for L-sorbose than for D-fructose, even though a *cis* pair of hydroxyl groups is present on carbon atoms 4 and 5 for D-fructopyranose but not for L-sorbopyranose, is strong evidence that with ketoses the accumulation of hydroxyl groups in the vicinity of the reducing carbon atom is the main factor in the formation of the boric acid complexes. Evidently this accumulation is of a particularly favorable arrangement in the case of L-sorbose.

THE HEXITOLS AND SOME OF THEIR DERIVATIVES

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Contents

Introduction

The story of the hexitols begins with the discovery of D-mannitol by Proust in 1806.[1] So far as the writers are aware, a review of the hexitols has not appeared previously even though the history of these compounds

[a] Deceased, Oct. 3, 1946.

(1) M. Berthelot, *Ann. chim. phys.*, [3] **47**, 297 (1856).

of widespread natural occurrence and of some importance industrially extends over nearly a century and a half. In spite of the early discovery of the naturally occurring hexitols (several are older than the corresponding hexoses), the synthesis of others nearly half a century ago, and the similarity of their chemistry to that of the sugars, their study has lagged behind that of other carbohydrate substances until recently, when three of the hexitols became available through commercial production. Early work on these compounds was carried out largely in the laboratories of Berthelot, Bertrand, Lobry de Bruyn and Emil Fischer in the nineteenth or early twentieth centuries. The field remained dormant until a decade ago when the study of anhydro and acetal derivatives was resumed with vigor. The anhydro derivatives are not included in the present review and the acetal derivatives will be considered only partially, though most of them will be included in the tables on pages 229–241. [Reviews of the general chemistry of anhydrides and acetals of polyhydric alcohols, including the hexitols, are scheduled for later volumes of the *Advances in Carbohydrate Chemistry*—The Editors.]

I. Occurrence and Preparation

There are ten hexitols, consisting of four pairs of optical enantiomorphs and two meso forms, that are predicted on the basis of the LeBel-van't Hoff theory of the asymmetric carbon atom. They may be regarded as reduction products of the hexoses and are now nearly always prepared by reducing or hydrogenating the hexoses. Sixteen aldohexoses give rise to only ten hexitols. Thus sorbitol[2] may be obtained either from D-glucose or L-gulose. Similarly, D-talitol is produced either from D-talose or D-altrose. The two meso hexitols, dulcitol and allitol (allodulcitol), are obtained from the appropriate hexoses of either steric series.[2a] The reduction of a ketose, through creation of a new asymmetric center, gives two hexitols.

Sorbitol, D-mannitol, L-iditol and dulcitol are found in plant sources. Synthetic preparation is simpler than isolation. The six other isomers

(2) Sorbitol may be classed as either of the D or the L series. C. S. Hudson has discussed the factors affecting this nomenclature in Vol. I of this series, pp. 14–15. The historical precedent is in favor of D-sorbitol, but there has been considerable confusion as to which designation is preferable. We prefer to regard sorbitol as a trivial name for the naturally occurring isomer, without designating it as D- or L-sorbitol. The enantiomorph is then either L-glucitol or D-gulitol and the name sorbitol is reserved exclusively for the natural isomer. When theoretical considerations require an assignment to a steric series, sorbitol may be called either D-glucitol or L-gulitol, whichever is apt.

(2a) See also C. S. Hudson, *Advances in Carbohydrate Chemistry*, **3**, 4 (1948).

are not known to occur naturally, but have been synthesized in crystalline condition.

1. *Sorbitol*

Sorbitol is found in many common fruits, particularly those of the *Rosaceae* family and of the genera *Pyrus*, *Sorbus*, *Photinia*, *Crataegus*, *Pyracantha* and *Cotoneaster*. The richest sources are the *Sorbus* and *Crataegus* species, especially the rowan berries of the European mountain ash tree (*Sorbus aucuparia*), the service berries of *Sorbus domestica*, and the fruits (haws) of the hawthorn (*Crataegus oxyacantha* L.); the fresh fruit of such trees often contains 5 to 10% sorbitol. It is also found in algae, oak galls and the tobacco plant. It occurs to a much less extent in grapes than in other wine fruits so that a sorbitol assay has been proposed as a means of detecting adulteration of grape wines with apple cider or other fruit wines[3]; it is claimed that as little as 5% adulteration may be detected. The sorbitol contents of a considerable number of species from several genera of the *Rosaceae* family have been determined by Strain[4] through use of a procedure which he discovered in the course of his[5] detection of sorbitol in the Christmas or toyon berries of a native Californian shrub or small tree (*Photinia arbutifolia* Lindl.). He found that although sorbitol could be isolated directly by older procedures it was more conveniently obtained by means of a crystalline molecular compound with pyridine. This compound is useful in isolating or purifying sorbitol as it crystallizes well and the pyridine is readily removed to leave crystalline sorbitol. Such a complex has not been reported to be formed from other hexitols, although one is formed with 2-desoxysorbitol (*syn.* 2-desoxy-D-mannitol) (see later reference 11*a*). Earlier isolations from a great many other species are found in the literature. Where the sorbitol content is low, the formation of insoluble benzylidene compounds is of value.

Sorbitol can be made by the reduction of three naturally occurring hexoses, D-glucose, D-fructose and L-sorbose. D-Mannitol and L-iditol, respectively, are concurrently produced from the ketoses. However, D-glucose, because of its greater availability, is the only practical source.

The first recorded reduction of D-glucose to sorbitol was in 1890 by Meunier[6] who used sodium amalgam. Ipatieff[7] reported the first catalytic hydrogenation of D-glucose to sorbitol. Since then there have been

(3) J. Werder, *Mitt. Lebensm. Hyg.*, **20,** 7, 245 (1929).
(4) H. H. Strain, *J. Am. Chem. Soc.*, **59,** 2264 (1937).
(5) H. H. Strain, *J. Am. Chem. Soc.*, **56,** 1756 (1934).
(6) J. Meunier, *Compt. rend.*, **111,** 49 (1890).
(7) V. Ipatieff, *Ber.*, **45,** 3218 (1912).

numerous other descriptions in which various catalysts and both high and low hydrogen pressures were used.

Glattfeld and Schimpff[8] found that the hydrogenation of D-glucono-γ-lactone over platinum catalyst, under certain conditions, gave a 41% yield of sorbitol, whereas under similar conditions the δ-lactone gave no sorbitol. D-Glucose is disproportionated to sorbitol and D-gluconic acid in the presence of Raney nickel and alkali.[9] This catalyzed Cannizzaro reaction has been applied to D-galactose as well as to noncarbohydrate aldehydes.

The superiority of catalytic hydrogenation of the readily available D-glucose as a laboratory method and the commercial availability of sorbitol has rendered the earlier reductions by sodium amalgam of historical interest only.

Commercially, electrolytic reduction and catalytic hydrogenation are used. The raw material may be crystalline D-glucose or hydrolyzed cane molasses (high-test molasses). The electrolysis is carried out in alkaline solution,[10] so that D-mannitol and several other isomers and related compounds are obtained because of the isomerizing effect of the alkaline catholyte.[11] The reduction may require from one to two weeks. The catalytic hydrogenation of D-glucose gives a product substantially free of isomers. It is carried out in neutral solution at high pressures, using a reduced nickel catalyst.

2. D-*Mannitol*

D-Mannitol is the most widely distributed of the naturally occurring hexitols and is found among the following groups: mannas, brown (but not green) marine algae, fruits, vegetables, grasses, flowers of herbs and trees, lichens and fungi. The manna ash, *Fraxinus Ornus*, the manna of which contains 30–50% D-mannitol, was formerly cultivated in Sicily for its D-mannitol, which was used as a laxative. It was then the only commercial source of the hexitol. Other hexitols were not then articles of commerce. The manna of the plane tree, *Platanus orientalis*, contains 80–90% D-mannitol. Several fungi contain 20%. Molasses from sugar cane which has been injured by freezing may contain as much as 15%

(8) J. W. E. Glattfeld and G. W. Schimpff, *J. Am. Chem. Soc.*, **57**, 2204 (1935).

(9) M. Delépine and A. Horeau, *Compt. rend.*, **204**, 1605 (1937).

(10) R. L. Taylor, *Chem. Met. Eng.*, **44**, 588 (1937); H. J. Creighton, *Trans. Electrochem. Soc.*, **75**, 389 (1939); *Can. Chem. Process Inds.*, **26**, 690 (1942).

(11) (*a*) M. L. Wolfrom, M. Konigsberg, F. B. Moody and R. M. Goepp, Jr., *J. Am. Chem. Soc.*, **68**, 122 (1946); (*b*) M. L. Wolfrom, F. B. Moody, M. Konigsberg and R. M. Goepp, Jr., *ibid.*, **68**, 578 (1946); (*c*) M. L. Wolfrom, B. W. Lew and R. M. Goepp, Jr., *ibid.*, **68**, 1443 (1946); (*d*) M. L. Wolfrom, B. W. Lew, R. A. Hales and R. M. Goepp, Jr., *ibid.*, **68**, 2342 (1946).

D-mannitol,[12] although normal molasses contains only a negligible amount.[13] It apparently arises from anaerobic fermentation. D-Mannitol is also present in silage and sauerkraut.

D-Mannitol, because of its high tendency to crystallize, is readily isolated from the various mannas in which it occurs. Although vegetable ivory nut represents a readily available source of mannan and ultimately D-mannose and D-mannitol, the process of producing the hexitol from this source cannot compete economically with that of obtaining it (along with sorbitol) from the alkaline electrolysis of D-glucose,[10] or from the hydrogenation of hydrolyzed molasses. D-Mannitol is readily separated from the sorbitol by crystallization from aqueous ethanol, in which the sorbitol is soluble. Several bacteria and molds, acting on a variety of substrates, produce D-mannitol, but their action does not compete with chemical methods, either industrially or in the laboratory.

3. *Dulcitol*

Dulcitol is found in various mannas. The manna of *Gymnosporia diflexa* Sprague is reported to contain 54% dulcitol.[14] It is also present in the "wahoo" or "burning bush," *Evonymus atropurpureus*, and in seaweeds. Xylose *torula* (yeast) contains about 1% dulcitol, dry basis.[15] The oldest historical source has been Madagascar manna. It was first prepared synthetically by Bouchardat[16] through the sodium amalgam reduction of D-galactose.

Dulcitol is no longer obtained in practice from Madagascar manna or other natural sources, but is produced by the catalytic hydrogenation of D-galactose, or, along with sorbitol, of hydrolyzed lactose; because of its lower solubility in water it is readily separated from the sorbitol.

4. L-*Iditol*

L-Iditol (sorbiérite), the last of the four naturally occurring hexitols, occurs in service berries along with sorbitol. After removing sorbitol from the juice by fermentation to L-sorbose with the sorbose bacterium, *Acetobacter xylinum*, the L-iditol is crystallized as a benzylidene derivative.[17] It has been prepared by the action of sodium amalgam on

(12) C. F. Walton, Jr. and C. A. Fort, *Ind. Eng. Chem.*, **23**, 1295 (1931).

(13) W. W. Binkley, Mary G. Blair and M. L. Wolfrom, *J. Am. Chem. Soc.*, **67**, 1789 (1945).

(14) J. R. Furlong and L. E. Campbell, *Proc. Chem. Soc.* (London), **29**, 128 (1913).

(15) H. Fink and F. Just, *Biochem. Z.*, **296**, 306 (1938).

(16) G. Bouchardat, *Compt. rend.*, **73**, 199 (1871), *Ann. chim. phys.*, [4] **27**, 68 (1872).

(17) G. Bertrand, *Ann. chim. phys.*, [8] **3**, 181 (1904).

L-idose[18] or L-sorbose.[19,20] More recently it has been prepared by the more convenient hydrogenation of these sugars.[21–23]

5. L-*Glucitol*

L-Glucitol (D-gulitol), the enantiomorph of sorbitol, has been obtained by the action of sodium amalgam on D-gulonolactone[24] or, along with D-iditol, on D-sorbose.[20]

While not significant as a preparative method, it is interesting to note its isolation, in very low yield, from the alkaline electrolysis of D-glucose. It was crystallized in the form of D,L-glucitol, identical with the product obtained by the crystallization of a mixture of equal parts sorbitol and L-glucitol (from the catalytic hydrogenation of D-gulose).[11d] Such a transformation apparently involved the enolization of D-glucose down through the 3,4-enediol, although the possibility of resynthesis of a six-carbon chain from smaller fragments is not precluded. This was the only completely inverted product isolated.

6. L-*Mannitol*

L-Mannitol has been prepared by the reduction of L-mannosaccharodilactone[24a] or L-mannose.[25] By far the most convenient procedure is that used by Baer and Fischer[26] for their preparation of L-glyceraldehyde by the oxidative cleavage of 1,2:5,6-diisopropylidene-L-mannitol with lead tetraacetate. L-Arabinose was converted to L-mannonolactone by the cyanohydrin synthesis and this was hydrogenated over platinum oxide to the desired L-mannitol. High hydrogen pressures, rather than low as usually employed with this catalyst, were used.

7. D-*Iditol*

D-Iditol has been prepared by the reduction of D-idonolactone.[18]

(18) E. Fischer and I. W. Fay, *Ber.*, **28**, 1975 (1895).

(19) G. Bertrand, *Bull. soc. chim.*, [3] **33**, 264 (1905); G. Bertrand and A. Lanzenberg, *ibid.*, [3] **35**, 1073 (1906).

(20) C. A. Lobry de Bruyn and W. Alberda van Ekenstein, *Rec. trav. chim.*, **19**, 1 (1900).

(21) A. S. Meyer and T. Reichstein, *Helv. Chim. Acta*, **29**, 152 (1946).

(22) G. Arragon, *Compt. rend.*, **205**, 735 (1937).

(23) F. B. Cramer and E. Pacsu, *J. Am. Chem. Soc.*, **59**, 1467 (1937).

(24) E. Fischer and R. Stahel, *Ber.*, **24**, 528 (1891).

(24*a*) H. Kiliani, *Ber.*, **20**, 2710 (1887).

(25) E. Fischer, *Ber.*, **23**, 370 (1890).

(26) E. Baer and H. O. L. Fischer, *J. Am. Chem. Soc.*, **61**, 761 (1939).

8. D-*Talitol*

D-Talitol has been prepared by the reduction of D-talonolactone[27] or D-talose.[28] Recently, an improved method of preparation, the hydrogenation of D-altrose in high yield, has been described.[29]

9. L-*Talitol*

L-Talitol has been synthesized by the catalytic hydrogenation of L-altrose.[30] It was the last of the ten isomeric hexitols to be synthesized.

10. *Allitol*

Allitol (allodulcitol) was first synthesized by Lespieau and Wiemann by a novel method, which does not involve the use of a preformed carbohydrate as starting material. The synthesis of allitol, D,L-mannitol, and dulcitol by the oxidation of divinylglycol or 1,2,5,6-tetrahydroxyhexene-3 is described by Lespieau in volume II of this series. The allitol prepared from divinyl glycol is identical with that obtained by the hydrogenation of D-allose.[31] Catalytic hydrogenation of *keto*-D-psicose pentaacetate, followed by acetylation, gives allitol hexaacetate.[11c] Allitol is also isolated in minor amounts from the commercial electro-reduction of D-glucose. The 2,3-enediol of D-glucose would tautomerize to D-psicose, which would be reduced to allitol and D-talitol. However, D-talitol was not found.[11c]

It is evident that syntheses of the Lespieau type can lead to a great number of polyhydroxy compounds. Wiemann[32] has prepared compounds such as $CH_3CH_2(CHOH)_6CH_2CH_3$, which he calls "diethyl mannitol." It would seem better to designate this substance a tetradesoxy decitol or decane hexol since its configuration is not known with certainty. Apparently this type of synthesis leads to symmetrical arrangements of hydroxyl groups, since allitol, dulcitol and D,L-mannitol are the only hexitols that were identified as products.

11. *Improvements in Synthesis*

The recent work of Fischer and collaborators on the reaction of nitromethane with aldoses will make the synthesis of some hexitols much

(27) E. Fischer, *Ber.*, **27**, 1524 (1894).
(28) G. Bertrand and P. Bruneau, *Bull. soc. chim.*, [4] **3**, 495 (1908).
(29) R. M. Hann, W. T. Haskins and C. S. Hudson, *J. Am. Chem. Soc.*, **69**, 624 (1947).
(30) F. L. Humoller, M. L. Wolfrom, B. W. Lew and R. M. Goepp, Jr., *J. Am. Chem. Soc.*, **67**, 1226 (1945).
(31) Marguerite Steiger and T. Reichstein, *Helv. Chim. Acta*, **19**, 184 (1936).
(32) J. Wiemann, *Ann. chim.*, [11] **5**, 267 (1936).

simpler. For example, L-mannose and L-glucose are made from readily available L-arabinose much more easily than by the older cyanohydrin synthesis.[33] These aldoses can be hydrogenated to the corresponding hexitols. Obviously the difficulty in obtaining the rarer hexitols resolves itself into the difficulty in obtaining the rare aldoses or aldonic acids.

The superiority of catalytic hydrogenation over reduction with sodium or aluminum amalgam as a means of converting hexoses to hexitols is apparent. The yields approach or reach the theoretical and the isomerizing effect of the alkaline reducing agent is avoided. Electrolytic reduction is not suitable as a laboratory method.

II. Physical Properties

The hexitols are colorless crystalline solids of sweet taste and varying solubility in water. Sorbitol is very soluble in water and is quite hygroscopic, being used commercially as a humectant. In general, the hexitols may be recrystallized from solutions in the lower alcohols, but they are insoluble in most other common organic solvents.

The melting point of sorbitol has been reported over a wide range for many years. The precise work of Rose and Goepp[34] showed that sorbitol occurs in a stable and an unstable form (freezing points, 97.2° and 92.7°, respectively). This accounts for the discrepancies, since most preparations are probably mixtures of the two forms, which are interconvertible. A value of 89–93° is usually encountered.[5]

The optical activity of the hexitols is of a low order. It may be enhanced by the addition of various complex-forming salts, borax and ammonium molybdate being the ones most used. Since the amount of enhancement is a function of the relative proportions of polyol and "booster," it is regrettable that definite amounts of borax or molybdate have not always been reported in the past.

The melting points and rotations of the hexitols are given in Table I, page 219.

III. Chemical Properties

The chemical reactions of the hexitols are similar to those of the simple sugars, uncomplicated by the presence of a carbonyl group. The hexitols exhibit a higher order of stability to acid, alkali and heat. They are readily converted to stable anhydro products. These anhydrides are known as hexitans when one mole of water is removed and hexides when two moles are removed.

(33) J. C. Sowden and H. O. L. Fischer, *J. Am. Chem. Soc.*, **69**, 1963 (1947).

(34) R. S. Rose, Jr., and R. M. Goepp, Jr., paper presented at the meeting of the American Chemical Society, Baltimore, April, 1939.

TABLE I
Physical Properties of the Hexitols

Hexitol	*Melting point, °C.*	$[\alpha]_D$	*Temp., °C.*	*Concentration in H_2O, g./100 ml.*	*Reference*
Sorbitol					
Stable form	96.7–97.2	−1.9[a]	25	10	34
Unstable "	90.4–91.8				34
L-Glucitol	89–91	+1.7	29	3.6	11d
D-Mannitol	166	−0.21[b]	25	17.6	35
		−0.19	25	15	34
		−0.40	25	7	34
L-Mannitol	164				25
D-Iditol	73.5	+3.5	20	10	36
L-Iditol	73.5	−3.53	20	10	19, 37
D-Talitol	87–88	+3.2	20	1.78	28, 29
L-Talitol	87–88	−2.9	20	5	30
Dulcitol	188.5	Optically inactive			38
Allitol	150–151	" "			31
D,L-Glucitol	136–138	" "			11d
D,L-Mannitol	170	" "			25
D,L-Iditol	Unknown				
D,L-Talitol	95–96	" "			30

[a] $[\alpha]^{25}_D$ is +6.63 in presence of 2 parts $Na_4B_4O_7 \cdot 10H_2O$ (c, 10) and +30.93 in presence of 2 parts $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (c, 5).[34]

[b] $[\alpha]^{25}_D$ is +28.61 in presence of 2 parts $Na_4B_4O_7 \cdot 10H_2O$ (c, 10) and +19.1 in presence of 2 parts $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (c, 2).[34]

The hexitols form the typical derivatives of alcohols and glycols—esters, ethers, acetals and ketals, and metallic complexes. Because of the multiplicity of reactive groups and because of stereoisomerism, the number of *theoretically* possible derivatives of a given type is enormous. For example, D-mannitol on reacting with a mono reagent such as an etherifying agent or an acid, can form 35 different compounds:

3	possible	mono-substituted	D-mannitol	derivatives	
9	"	di-	"	"	"
10	"	tri-	"	"	"
9	"	tetra-	"	"	"
3	"	penta-	"	"	"
1	"	hexa-	"	"	"

(35) J. M. Braham, *J. Am. Chem. Soc.*, **41,** 1707 (1919).
(36) G. Bertrand and A. Lanzenberg, *Compt. rend.*, **143,** 291 (1906).
(37) G. Bertrand, *Bull. soc. chim.*, [3] **33,** 166 (1905); *Ann. chim. phys.*, [8] **10,** 450 (1907).
(38) C. N. Riiber, T. Sörenson and K. Thorkelsen, *Ber.*, **58,** 964 (1925).

It should be noted that the following positions are synonymous in D- or L-mannitol and D- or L-iditol: 1 and 6, 2 and 5, and 3 and 4. Sorbitol, because of lower optical symmetry, would form even more derivatives. The number of possible acetals is much smaller and that of metallic complexes still smaller. The number of various theoretical permutations of mixed ether-esters, ether-acetals, etc., utilizing readily available reagents, runs into the hundreds of thousands.

Tables listing those oxygen-substituted derivatives whose structure is known with a reasonable degree of certainty are given at the end of this article, pages 229–241.

1. *Esterification*

The inorganic esters of hexitols are not well characterized. Many are described in Beilstein. D-Mannitol hexanitrate is best known. It has found considerable use as a detonator for blasting caps since it is more resistant to shock, impact and friction, both in manufacturing operations and in the field, than detonators such as mercury fulminate or lead azide. It is in itself an explosive of strength equal to that of nitroglycerin. Pharmacologically it is a valuable vasodilator, acting similarly to nitroglycerin or amyl nitrite, but having a more gradual and sustained action. The pharmacological behavior of various hexitol and anhydrohexitol nitrates has been investigated.[39]

There are many reports of hexitol borates, but most of these are probably not true esters but complexes. Only two well-defined crystalline esters, 1-D-mannitol monoborate[40,41] and 2-D-mannitol monoborate[41] are known. The borate esters are unstable in water.

The organic esters have a greater order of stability, but it is difficult to prepare completely acylated compounds without concurrently anhydrizing the hexitol unless one uses acid anhydrides or chlorides. Early attempts to prepare higher aliphatic esters of D-mannitol resulted in the formation of mixtures of mannitans and mannides. It is for this reason that caution must be exercised in interpreting some of the work in the literature. The analytical values of the pure compounds and the mixtures are such that one cannot differentiate between them.

Although the partial esters of sorbitol and D-mannitol have long been known and are the most important derivatives commercially, very little is known of their structure. With pure fatty acids now obtainable in the

(39) J. C. Krantz, Jr., C. J. Carr, S. E. Forman and F. W. Ellis, *J. Pharmacol.*, **67**, 187, 191 (1939).

(40) J. J. Fox and A. J. H. Gauge, *J. Chem. Soc.*, **99**, 1075 (1911).

(41) W. H. Holst, paper presented at the meeting of the American Chemical Society, Baltimore, April, 1939.

laboratory and in view of the recent structural work on the anhydrohexitols, it may be that this situation will change. It is not worth while to enumerate here the large number of various esters that have been prepared. They are most certainly mixtures. An excellent review[42] exists on their preparation, properties and use. Few attempts to prepare pure fatty acid esters have been made. Bloor[43] prepared esters from several higher fatty acids and D-mannitol in order to compare their metabolism with that of the glycerides. His preparations were apparently homogeneous and it is highly probable that his mannitan and mannide esters were esters of 1,4-D-mannitan and 1,4:3,6-D-mannide (isomannide).

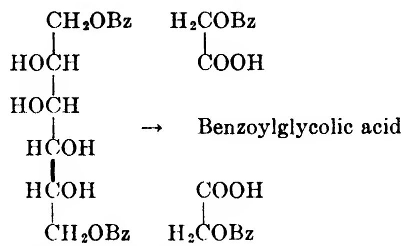
The saponification and carbon and hydrogen values of some fatty acid esters of hexitols and anhydrohexitols are so nearly the same that they do not permit ready differentiation. The determination of the hydroxyl content is essential on these substances, but it has not been widely adopted. Various methods are available, but those based on the acetylation of the material with pyridine–acetic anhydride, followed by hydrolysis of the excess anhydride and titration with alkali[43a] seem to be best adapted to esters of polyols. The ratio of saponification number (milligrams potassium hydroxide necessary to saponify one gram of material) to hydroxyl number (milligrams potassium hydroxide equivalent to acid to acetylate one gram of material) provides a convenient method for identifying the type of ester. For example, the ratio for a hexitol tetraester is 2, for a hexitan triester, 3, etc. This method is independent of the nature of the acyl portion and is therefore applicable to esters prepared from commercial grades of fatty acids or to mixed esters. It is not universally applicable because some esters will have a like ratio—for example, a hexitol triester and a hexitan diester both have a ratio of 1. Where the acyl portion is pure, the absolute values of the saponification and hydroxyl numbers often permit a differentiation between those compounds having the same ratio.

The hexaacetates of the hexitols are admirably suited for identification purposes, being easily crystallized, possessing sharp melting points and having higher specific rotations than the parent hexitols.

Since the hexitols are saturated aliphatic compounds, the asymmetric centers are generally assumed to have free rotation. Hence one cannot with certainty predict differences in reactivity between "*cis*" and "*trans*" pairs of hydroxyl groups on the basis of steric hindrance. Thus one cannot say that D-mannitol, because of its two pairs of "*cis*" hydroxyl

(42) H. A. Goldsmith, *Chem. Revs.*, **33**, 257 (1943).
(43) W. R. Bloor, *J. Biol. Chem.*, **7**, 427 (1910), **11**, 141, 421 (1912).
(43a) E. S. West, C. I. Hoagland and G. H. Curtis, *J. Biol. Chem.*, **104**, 627 (1934).

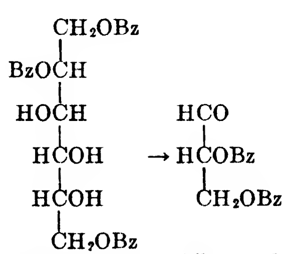
groups in the projection formula (which is a conventional representation) will be more difficult to esterify than, say, sorbitol. There is, however, sufficient difference between the reactivity of the primary and secondary hydroxyl groups of D-mannitol[44–46] and sorbitol[45,47] to permit the direct formation of 1,6-dibenzoates when treated with limited amounts of benzoyl chloride in pyridine. The structure of the D-mannitol ester was shown by its oxidation to benzoylglycolic acid by permanganate.[46] In the case of 1,6-dibenzoylsorbitol, it was shown that oxidation with lead



[1,6-Dibenzoyl-D-mannitol]

tetraacetate consumed three moles of oxidant and liberated no formaldehyde.[45] No other diester would lead to this combination.

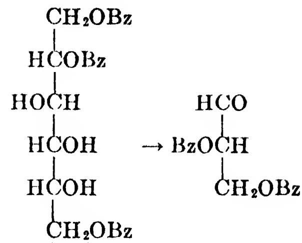
By-products of the above reactions are 1,2,6-tribenzoyl-D-mannitol[48] and 1,2,6-tribenzoylsorbitol.[45] The structure of the first was shown in two ways. Brigl and Grüner[48] converted it to the known 1,2,5,6-tetrabenzoyl-3,4-isopropylidene-D-mannitol. Hockett and Fletcher[45] oxidized it to dibenzoyl-D-glyceraldehyde with lead tetraacetate. This, and the fact that the rate of oxidation was like that of glycerol, not of ethylene glycol, established the structure. 1,2,6-Tribenzoylsorbitol consumed



1,2,6-(*syn.* 1,5,6-) Tribenzoyl-D-mannitol   Dibenzoyl-D-glyceraldehyde

two moles of oxidant, liberated no formaldehyde, and led to dibenzoyl-L-

(44) A. Einhorn and F. Hollandt, *Ann.*, **301**, 95 (1898).
(45) R. C. Hockett and H. G. Fletcher, Jr., *J. Am. Chem. Soc.*, **66**, 469 (1944).
(46) P. Brigl and H. Grüner, *Ber.*, **65**, 641 (1932).
(47) A. Müller, *Ber.*, **65**, 1055 (1932).
(48) P. Brigl and H. Grüner, *Ber.*, **66**, 931 (1933).



1,2,6-Tribenzoylsorbitol Dibenzoyl-L-glyceraldehyde

glyceraldehyde. This study has not been extended to other hexitols or acids, although several known diesters of D-mannitol may be 1,6-diesters, since they were prepared similarly to the dibenzoates (Table III, page 233).

2. *Etherification*

Aliphatic ethers have not been formed selectively, but Irvine and Patterson[49] found that, with silver oxide and methyl iodide, it was not possible to introduce the sixth methyl group, on hydroxyl 1, into partially methylated D-mannitol obtained by reducing methylated D-mannose. However, D-mannitol can be completely methylated with dimethyl sulfate and sodium hydroxide in carbon tetrachloride.[50]

Trityl chloride reacts more readily with primary than with secondary hydroxyl groups, so that it is possible to isolate 1,6-ditrityl ethers.[51]

Commercially, the polyalkylene oxide derivatives made by reacting ethylene oxide or propylene oxide with hexitols, or their partial esters, are the most important ethers.

3. *Reaction with Aldehydes and Ketones*

In contrast to the esters and ethers, many acetals and ketals are known. It is with these derivatives that most of the interesting structural work has been done.

Acetals of hexitols form preferentially according to a definite pattern elucidated by Hann and Hudson.[52] For the methylene or benzylidene acetals they make the following generalizations. (*1*) In the case of

(49) J. C. Irvine and Bina M. Patterson, *J. Chem. Soc.*, **105**, 915 (1914). The methylated D-mannose was prepared from α-methyl D-mannoside, which the authors thought to be furanoid. The ether was reported as 2,3,5,6-tetramethyl-D-mannitol, but, since the starting D-mannoside is now known to have been pyranoid, the ester must be 2,3,4,6-tetramethyl-D-mannitol.

(50) W. Freudenberg and J. T. Sheehan, *J. Am. Chem. Soc.*, **62**, 558 (1940).

(51) F. Valentin, *Collection Czechoslov. Chem. Commun.*, **3**, 499 (1931). See also later reference 132 and the review of trityl ethers by B. Helferich, *Advances in Carbohydrate Chem.*, **3**, 79 (1948).

(52) R. M. Hann and C. S. Hudson, *J. Am. Chem. Soc.*, **66**, 1909 (1944).

secondary hydroxyl groups of β position, a *cis* relationship (as defined below) is favorable to acetal formation, but a *trans* relationship is unfavorable; (*2*) in the case of secondary hydroxyl groups of γ position a *trans* relationship is favorable to acetal formation; (*3*) a primary and secondary alcohol group in β position are favorable to acetal formation. They use the expressions *cis* and *trans* in these generalizations to indicate the relative positions of secondary hydroxyl groups in the customary straight-chain stereo-formulas of the hexitols; thus the four secondary hydroxyl groups of allitol (see page 241 for its stereo-formula) have *cis* relationship in so far as the formula is concerned; in the case of L-iditol (see page 239 for its stereo-formula) the hydroxyl groups on carbon atoms 2 and 4 have *cis* relationship, and likewise the hydroxyl groups on carbon atoms 3 and 5, but the hydroxyl groups on carbon atoms 2 and 3 have *trans* relationship in the stereo-formula. In this use of *cis* and *trans*, the words apply to positions not with reference to a plane but rather with reference to the straight line that connects the carbon atoms in the customary stereo-formulas introduced by Emil Fischer.

According to von Vargha[53] the points of attachment of the first isopropylidene group in D-mannitol can be altered by the use of boric acid. Ordinarily the 3,4 isomer is obtained. However, in the presence of boric acid a 1,2-isopropylidene-4,5-monoborate is formed which is readily decomposed to 1,2-isopropylidene-D-mannitol.

4. *Metallic Complexes*

Soluble complexes are formed with metallic oxides, especially in the presence of alkali hydroxides. The strong tendency of hexitols to dissolve metallic oxides presents considerable technical difficulty in their manufacture and for this reason glass, rubber or stainless-steel equipment is used. In some instances well defined complexes can be isolated, particularly with alkaline earth oxides or mixtures with ferric oxide.[54] These complexes absorb carbon dioxide and water and are unstable in dilute aqueous solution. Their structures are not established, but are inferred from analytical and physical measurements. Diehl[55] has reviewed the subject.

The complexes with boric acid and borates have been of more interest. The ability of D-mannitol to render boric acid more acidic is well known and forms the basis of an analytical method allowing boric acid to be titrated as a monobasic acid. The boric acid complexes have been

(53) L. von Vargha, *Ber.*, **66**, 1394 (1933).

(54) A. Grün, *Monatsh.*, **37**, 205 (1916); W. Traube and F. Kuhbier, *Ber.*, **65**, 187 (1932); W. Traube, F. Kuhbier and H. Härting, *Ber.*, **66**, 1545 (1933).

(55) H. Diehl, *Chem. Revs.*, **21**, 39 (1937).

investigated from a physicochemical point of view by Böeseken.[56] The salts of these complexes enhance the specific rotations of the hexitols.

Recently Isbell and coworkers[56a] have published the results of an extensive study of the behavior of solutions of sorbitol and D-mannitol in the presence of tetraborates. They found that sorbitol appears to form three complex borate compounds, whereas D-mannitol forms only two. Since the specific rotation in the tetraborate-D-mannitol system is a function of the ratio of the components and is independent of concentration at constant tetraborate-D-mannitol ratios, D-mannitol can be determined quantitatively by this method. However, sorbitol cannot be determined this way because the change in observed rotation at constant tetraborate concentration shows a reversal with increasing amounts of sorbitol.

The potentiating effects of various polyols on boric acid have been investigated, notably by Böeseken,[57] Mellon and Morris[58] and Krantz and coworkers.[59]

The idea is prevalent that D-mannitol has a greater potentiating effect than sorbitol because its two pairs of "*cis*" hydroxyl groups are more likely to undergo complex formation. This erroneous idea may have arisen by faulty analogy with α- and β-glucose, which, however, have the arrangement in space fixed by a ring. Böeseken,[57] in his paper on the configuration of α- and β-glucose, emphasizes that the hexitols are an entirely different case. Indeed, measurements of conductivity or alkaline titrations of solutions of the various hexitols and boric acid show only small differences in their effect. D-Mannitol has found favor in the analytical titration of boric acid because it was for many years the only readily available pure crystalline hexitol, not because of any intrinsic superiority as a potentiating agent.

Complex D-mannitol borates are used in electrolytic condensers. Molybdic and arsenic acids also form association complexes with hexitols which increase the acidity of the inorganic acids. Rotational exaltation is obtained with the salts of these acids.

(56) J. Böeseken, N. Vermaas, W. H. Zaayer and J. L. Leefers, *Rec. trav. chim.*, **54,** 853 (1935). See also the review by J. Böeseken in this volume of the *Advances in Carbohydrate Chemistry.*

(56*a*) H. S. Isbell, J. F. Brewster, Nancy B. Holt and Harriet L. Frush, *J. Research Nat. Bur. Standards*, **40,** 129 (1948).

(57) J. Böeseken, *Ber.*, **46,** 2612 (1913).

(58) M. G. Mellon and V. N. Morris, *Ind. Eng. Chem.*, **16,** 123 (1924).

(59) J. C. Krantz, Jr., Margarethe Oakley and C. J. Carr, *J. Phys. Chem.*, **40,** 151 (1936); J. C. Krantz, Jr., C. J. Carr and Frances F. Beck, *ibid.*, **40,** 927 (1936); F. K. Bell, C. J. Carr, W. E. Evans and J. C. Krantz, Jr., *ibid.*, **42,** 507 (1938); F. K. Bell, C. J. Carr and J. C. Krantz, Jr., *ibid.*, **44,** 862 (1940).

5. *Oxidation*

By far the most important oxidation of the hexitols is their specific biochemical transformation to ketoses. The history of this reaction dates from the fortuitous discovery of L-sorbose in mountain ash berries by Pelouze[60] in 1852. It was not until twenty years later that Boussingault[61] showed that it had arisen by bacterial oxidation of sorbitol.

This reaction was investigated extensively by Bertrand, and his generalization[17] on the specificity of this oxidation has come to be known as "Bertrand's Rule." If the first and second asymmetric carbon atoms of a polyhydric alcohol have hydroxyl groups of like configuration, the substance will be converted to a ketose by the "sorbose bacterium." *Acetobacter xylinum,* the organism that was studied by Bertrand, oxidizes sorbitol to L-sorbose, D-mannitol to D-fructose, and allitol to L-psicose.[62] Enantiomorphs of these have not been tested, but from studies on the pentitols, we find that both enantiomorphous forms are not attacked. There is also a species specificity. *Acetobacter suboxydans* oxidizes some polyols of the series not oxidized by *A. xylinum,* and vice versa.[63] Other species of *Acetobacter* oxidize polyols to ketoses, but have been studied less extensively. It is evident that the action of the organisms must be tested on more of the polyols before Bertrand's Rule can be extended to predict which optical form of the hexitols will be attacked. This oxidation is of great industrial importance because of the requirement for large amounts of L-sorbose for the synthesis of ascorbic acid.

Chemical oxidations have proved to be inferior to biochemical in the production of ketoses from hexitols. The only clear-cut chemical oxidation is that by Sullivan,[64] who transformed sorbitol to L-sorbose by the chromic acid oxidation of 6-benzoyl-1,3:2,4-diethylidene sorbitol to 1-benzoyl-3,5:4,6-diethylidene-*keto*-L-sorbose. Removal of the blocking groups gave crystalline L-sorbose. Chemical oxidation of hexitols containing unprotected hydroxyl groups is unfruitful because of the lack of specificity of the oxidizing agent. Several sugars are usually formed and the yield of the desired product is low.

Oxidation of the hexitols to saccharic acids is accomplished in the same manner as the oxidation of the aldohexoses. This is mainly of theoretical interest since the same products are obtained from the generally more

(60) J. Pelouze, *Ann. chim. phys.*, [3] **35**, 222 (1852).

(61) J. Boussingault, *Compt. rend.*, **74**, 939 (1872).

(62) Marguerite Steiger and T. Reichstein, *Helv. Chim. Acta*, **18**, 790 (1935).

(63) R. M. Hann, Evelyn B. Tilden and C. S. Hudson, *J. Am. Chem. Soc.*, **60**, 1201 (1938).

(64) W. R. Sullivan, *J. Am. Chem. Soc.*, **67**, 837 (1945).

accessible aldoses or aldonic acids; an exception is the preparation of L-idosaccharic acid by the nitric acid oxidation of L-iditol.[64a]

D-Mannitol, sorbitol and dulcitol are oxidized at the same rate by lead tetraacetate.[65]

IV. Analysis

The separation of mixtures of hexitols has long been a difficult problem. The removal of sorbitol from L-iditol by bacterial action is a classical example. Destruction of one component as a means of separation is drastic and is applicable to only a few mixtures. Even from an analytical point of view, separation has been difficult. The proportions of D-mannitol and sorbitol in the reduction products of D-fructose may be determined approximately by crystallizing and weighing the D-mannitol, but the amount of D-mannitol still in solution remains an unknown quantity.

The discovery that the hexitols can be separated by chromatography on clay[66] or synthetic[67] columns was therefore of great interest to those working with these compounds. In the relatively short time that it has been known, the technique has been much used. The proportion of hexitol in rather complex mixtures containing sugars, sugar acids and anhydrohexitols may be found by this method. The acetates may also be used. The technique is readily mastered and it can be used on samples weighing only a few milligrams. Furthermore, the same sample may be used again if an unsuitable developer is chosen initially. It is the only method known that has general applicability. It is limited to relatively small samples so that its utility as a means of isolating hexitols for purposes other than analysis is somewhat less. Older methods of analysis of mixtures, based on optical activity (usually enhanced) are of little value.

The benzylidene or *m*-nitrobenzylidene derivatives, because of their insolubility, are well suited for isolation of small quantities of hexitols. The difficulty in obtaining completely or partially benzylidenated products free from one another limits their usefulness for characterization, since varying melting points are obtained. *Cis-trans* isomerization is another complication. Conversion to the acetates obviates these difficulties.

(64*a*) W. G. M. Jones and L. F. Wiggins, *J. Chem. Soc.*, 363 (1944).

(65) R. C. Hockett, Margaret T. Dienes, H. G. Fletcher, Jr. and H. E. Ramsden, *J. Am. Chem. Soc.*, **66**, 467 (1944).

(66) B. W. Lew, M. L. Wolfrom and R. M. Goepp, Jr., *J. Am. Chem. Soc.*, **68**, 1449 (1946).

(67) L. W. Georges, R. S. Bower and M. L. Wolfrom, *J. Am. Chem. Soc.*, **68**, 2169 (1946).

The hexitols may be determined quantitatively by periodate oxidation.[68] Either the amount of oxidant consumed or the amount of acid or formaldehyde liberated may be determined. This method will not

$$CH_2OH(CHOH)_4CH_2OH + 5\,IO_4^- \rightarrow 4\,HCO_2H + 2\,HCHO + 5\,IO_3^- + H_2O$$

distinguish between the different hexitols and will give the total amount of hexitol present. For differentiation, recourse to chromatographic methods is necessary.

(68) E. L. Jackson in "Organic Reactions" (R. Adams, editor), John Wiley and Sons, New York, Vol. II, p. 341 (1944).

TABLE II

Derivatives of Sorbitol ($CH_2OH-\underset{OH}{\overset{H}{C}}-\underset{OH}{\overset{H}{C}}-\underset{H}{\overset{OH}{C}}-\underset{OH}{\overset{H}{C}}-CH_2OH$)

(6) (5) (4) (3) (2) (1)

(The following abbreviations are used in this and the subsequent tables: Ac for acetyl, Pr for propionyl, Bu for butyryl, Bz for benzoyl, Ts for tosyl, My for methylene, Ed for ethylidene, Bd for benzylidene, Fd for furfurylidene, Id for isopropylidene, Me for methyl, Et for ethyl, Be for benzyl, Tr for trityl and Az for azoyl. Where the linkages of acetals and ketals are known, they are shown by different type fonts.)

Substituent						*Melting point, °C.*	[α]D	*Solvent*	*References*
6	*5*	*4*	*3*	*2*	*1*				
Ac	Ac	Ac	Ac	Ac	Ac	99	+10.0	Chloroform	68*a*, *b*
Pr	Pr	Pr	Pr	Pr	Pr	Sirup	+14.1	Chloroform	69
Bu	Bu	Bu	Bu	Bu	Bu	Sirup	+17.4	Chloroform	69
Bz	Bz	Ac	Ac	Ac	Ac	96–97	+14.4	Chloroform	70
Bz	—	—	—	Bz	Bz	148	−11.1	Chloroform	45
Bz	—	—	—	—	Bz	141	+ 1.85	Pyridine	44, 47
MY	MY	*My*	My	*My*	My	212–216	−30.8	Chloroform	71–73
Ed	Ed	Ed	Ed	Ed	Ed	174–176	—		74
Ed	Ed	Ed	Ed	Ed	Ed	96–97	−21.6	Water	64
BD	BD	*Bd*	Bd	*Bd*	Bd	203	+26.9	Chloroform	75, 76
Dimorph						190–191			76
FD	FD	*Fd*	Fd	*Fd*	Fd	186–187	+19.7	Chloroform	77
Id	Id	Id	Id	Id	Id	45–46	+14.2	Abs. alc.	5, 78
Id	*Id*	Bd	Id	Bd	Id	131–132	+26.7	Chloroform	79, 80
—	—	*My*	My	*My*	My	174–175	−29.6	Chloroform	72, 73
—	*My*	My	*My*	My	—	192–193	+41.5	Water	81, 82
—	—	*Ed*	Ed	*Ed*	Ed	212–214	−11.1	Water	74, 83
—	—	*Bd*	Bd	*Bd*	Bd	219–221	+21.6	Pyridine	70, 76, 84
—	*Bd*	Bd	*Bd*	Bd	—	208	−14.8	Acetone	84*a*
Fd	*Fd*	Fd	—	Fd	—	202–203	—		77
1,3:2,4-Dimethylene-5,6-acrylidene						160	+280	Chloroform	84*a*
Id	Id	Bd	—	Bd	—	179	+19.0	Chloroform	79, 80, 85
—	—	My	—	My	—	163–164	− 9.8	Water	72, 73
—	—	Bd	—	Bd	—	176–177	− 1.1	Water	6, 79, 80
						195–196	+ 5.6	Water	5, 76, 86
—	—	Fd	—	Fd	—	192–193	—		77
Tri-(*o*-nitrobenzylidene)						142–146	—		87
Stable form						213.5	—		88
Tri-(*m*-nitrobenzylidene)						168	—		88
Tri-(*o*-chlorobenzylidene)						217	—		88
Di-(*m*-nitrobenzylidene)						228.5	—		88, 89
Di-(*p*-nitrobenzylidene)						150	−58	Chloroform	75
Mono-(*o*-nitrobenzylidene)						181	—		88

TABLE II (*Continued*)

Substituent 6	5	4	3	2	1	*Melting point, °C.*	$[\alpha]_D$	*Solvent*	*References*
Mono-(*m*-nitrobenzylidene)						180	—		88
Mono-(*p*-nitrobenzylidene)						204.5	—		89
Mono-(*o*-chlorobenzylidene)						170	—		88
Mono-(2,6-dichlorobenzylidene)						204.5	—		88
Mono-(2-nitro-5-chlorobenzylidene)						250.5	—		88
Me	Me	Me	Me	Me	Me	Sirup	+ 1.97	No solvent	50
Hexaallyl						Sirup	+ 6.56	Ethanol	90
Me	Me	Me	Me	Me	—	Sirup	+47	Chloroform	91
Me	Me	—	Me	Me	Me	Sirup	−10.1	Abs. alc.	92
Me	Me	—	Me	Me	—	Sirup	− 6.2	Water	93, 94
Me	—	Me	Me	Me	—	Sirup	+10.3	Ethanol	50
							+10.8	Water	94*a*
Me	—	Me	Me	—	—	Sirup	+15.1	Water	94*a*
Me	—	Me	—	Me	—	Sirup	+13.1	Water	94*a*
Me	—	—	Me	Me	—	Sirup	+ 3.4	Water	94*a*
—	—	Me	Me	Me	—	64	+ 8.3	Water	94*a*
—	—	—	Me	Me	—	Sirup	+13.0	Water	94*a*
Tr	—	—	—	—	Tr	72–83	− 7.8	Benzene	51
—	—	—	Me	—	—	Sirup	—		95
Me	—	—	—	—	—	Sirup	+ 4.27	Water	96
Ac	Ac	*My*	My	*My*	My	135–136	−12.8	Chloroform	72, 73
Bz	Bz	*My*	My	*My*	My	134–135	−54.8	Chloroform	72, 73
Me	Me	*My*	My	*My*	My	193–194	−23.8	Chloroform	73
Bz	—	*My*	My	*My*	My	195–197	−15.9	Chloroform	73
Tr	—	*My*	My	*My*	My	194	− 8	Chloroform	73
Ts	—	*My*	My	*My*	My	160–161	−10.0	Chloroform	97
Ac	*My*	My	*My*	My	Ac	114–115	+ 6.6	Chloroform	81
Bz	*My*	My	*My*	My	Bz	158–159	+18.7	Chloroform	81, 82
Ts	*My*	My	*My*	My	Ts	97–98	+ 4.6	Chloroform	81, 82
1,6-Dimethacrylyl-2,4:3,5-dimethylene						90	+93.2	Chloroform	84*a*
Tr	*My*	My	*My*	My	Tr	209–210	+12.0	Chloroform	82
Me	*My*	My	*My*	My	Me	43	+ 9.4	Chloroform	82
Ac	Ac	My	Ac	My	Ac	150–151	− 1.5	Chloroform	72, 73
Ts	Ac	My	Ac	My	Ts	130–131	− 4.8	Chloroform	98
3-Methyl dimethylene						133–134	−10.1	Water	95
Ts	—	My	—	My	Ts	129–130	− 2	Chloroform	73, 98
Tr	—	My	—	My	Tr	112–115	—		73
2,4-Methylene tribenzoyl						154	−10	Chloroform	73

TABLE II (*Continued*)

Substituent 6	5	4	3	2	1	*Melting point, °C.*	$[\alpha]_D$	*Solvent*	*References*
Bz	Bz	*Ed*	Ed	*Ed*	Ed	124	−34.0	Chloroform	64
Ts	Ts	*Ed*	Ed	*Ed*	Ed	172	−12.8	Chloroform	64
Me	Me	*Ed*	Ed	*Ed*	Ed	Sirup	− 9.8	Chloroform	73
Ts	Ac	*Ed*	Ed	*Ed*	Ed	140	+ 6.0	Chloroform	99
—	Bz	*Ed*	Ed	*Ed*	Ed	202	−119	Chloroform	97*a*
Bz	—	*Ed*	Ed	*Ed*	Ed	167	+ 5.9	Chloroform	64
Ts	—	*Ed*	Ed	*Ed*	Ed	92	+11.5	Chloroform	64, 96
Me	—	*Ed*	Ed	*Ed*	Ed	70	+ 4.5	Chloroform	96
Tr	—	*Ed*	Ed	*Ed*	Ed	92	− 5.4	Chloroform	97*a*
Tr	Bz	*Ed*	Ed	*Ed*	Ed	213–214	−21.9	Chloroform	97*a*
Ac	Ac	*Bd*	Bd	*Bd*	Bd	208–209	+ 4.1	Chloroform	70, 76
Bz	Bz	*Bd*	Bd	*Bd*	Bd	195–196	−41.5	Chloroform	70, 76
Ts	Ts	*Bd*	Bd	*Bd*	Bd	159–160	+ 1.2	Acetone	70, 76
Tr	Ac	*Bd*	Bd	*Bd*	Bd	186–187	−41.8	Ethyl acetate	70, 76
							−46.5	Chloroform	
Dimorph						117–119			70
Tr	—	*Bd*	Bd	*Bd*	Bd	182–183	+16.8	Ethyl acetate	70, 76
Dimorph						110–115			70
Bz	*Bd*	Bd	*Bd*	Bd	Bz	208	− 1.9	Chloroform	80
Ts	*Bd*	Bd	*Bd*	Bd	Ts	123–124	+ 7.8	Chloroform	99*a*
1,6-Dimethacrylyl-2,4:3,5-dibenzylidene						135	−26.2	Chloroform	84*a*
Ac	Ac	Bd	Ac	Bd	Ac	87–88	− 6.7	Chloroform	79, 80
Id	Id	Bd	Bz	Bd	Bz	118–119	—		79, 80
Tr	Ac	Bd	Ac	Bd	Tr	106–108	+21.3	Chloroform	80, 86
Ts	—	Bd	—	Bd	Ts	148	+17.8	Pyridine	80
Bz	—	Bd	—	Bd	Bz	172	−10.8	Pyridine	80
Tr	—	Bd	—	Bd	Tr	100–103	+20	Chloroform	86
Me	—	Bd	—	Bd	Ts	128	− 1.2	Pyridine	80
Tr	—	Fd	—	Fd	Tr	222–224	—		77
Me	Ac	Ac	Ac	Ac	Ac	105	+ 7.6	Chloroform	96
Az	Az	Az	Me	Me	Az	181	+104	Benzene	94*a*
Az	Az	Me	Me	Me	Az	85	+50	Benzene	94*a*
Me	Az	Az	Me	Me	Az	170	+30	Benzene	94*a*
Me	Az	Me	Az	Me	Az	61	−36	Benzene	94*a*
Me	Az	Me	Me	Az	Az	201	−86	Benzene	94*a*
Me	Az	Me	Me	Me	Az	159	−55	Benzene	94*a*
2,3,4,5,6-Pentamethyl-1-α-naphthylcarbamate						75–76	− 5	Chloroform	91

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TABLE III

Derivatives of D-*Mannitol* $\left(\underset{(6)}{CH_2OH}-\underset{\underset{(5)}{OH}}{\overset{H}{C}}-\underset{\underset{(4)}{OH}}{\overset{H}{C}}-\underset{\underset{(3)}{H}}{\overset{OH}{C}}-\underset{\underset{(2)}{H}}{\overset{OH}{C}}-\underset{(1)}{CH_2OH}\right)$

(Abbreviations are defined on page 229)

Substituent 6	5	4	3	2	1	*Melting point, °C.*	$[\alpha]_D$	*Solvent*	*References*
Ac	Ac	Ac	Ac	Ac	Ac	126	+ 25.0	Chloroform	68*a*, 119
Bz	Bz	Bz	Bz	Bz	Bz	149	+ 50.7	Chloroform	64, 100
Hexa-*p*-bromobenzoyl						96	+ 29.6	Chloroform	101
Hexagalloyl						Amorph.	+ 27.1	Ethanol	102
Hexacinnamoyl						99–100	+ 13.0	Chloroform	103
Hexa-(triacetylgalloyl)						Amorph.	—		102
Hexa-(tribenzoylgalloyl)						150	+ 19	Acetylene tetrachloride	104
Hexaphenylcarbamate						303	—		104*a*
Bz	Ac	Ac	Ac	Ac	Bz	126–127	+ 38.9	Chloroform	105, 106
Ts	Ac	Ac	Ac	Ac	Ts	119–120	+ 22.9	Chloroform	105
Ac	Ac	Ts	Ts	Ac	Ac	Sirup	− 0.8	Chloroform	107
Ac	Bz	Bz	Bz	Bz	Ac	146	+ 40.4	Chloroform	108
Bz	Ac	Ts	Ts	Ac	Bz	142	+ 55.9	Chloroform	109
Bz	Bz	Ac	Ac	Bz	Bz	127	+ 72.2	Chloroform	48
Bz	Ts	Ac	Ac	Ts	Bz	121	+ 56.4	Chloroform	109
Ts	Bz	Bz	Bz	Bz	Ts	166	+ 36.9	Chloroform	108
Bz	Bz	Ts	Ts	Bz	Bz	136–137	− 4.8	Chloroform	110
Bz	Bz	Ts	Bz	Ts	Bz	153	+ 41.6	Chloroform	111
Bz	Ts	Ts	Ts	Ts	Bz	159	+ 42.0	Chloroform	112
Ac	Ac	Ac	Ac	—	—	92	+ 31.6	Chloroform	53
—	Ac	Ac	Ac	Ac	—	123–125	+ 3	Chloroform	113
—	Bz	Bz	Bz	Bz	—	155, 145–147	− 0.5	Chloroform	108, 114
Bz	Bz	Bz	Bz	—	—	Amorph.	+ 52.6	Chloroform	53
Bz	Bz	—	—	Bz	Bz	122–123	+ 7.8	Acetylene tetrachloride	48, 115
Bz	—	Ts	Ts	—	Bz	145–146	+ 42.2	Chloroform	109
Bz	Ts	—	—	Ts	Bz	76–77	− 27.8	Chloroform	109
Bz	—	—	—	Bz	Bz	166–167	− 43.7	Pyridine	45, 106
—	—	Ac	Ac	—	—	139	+ 45.3	Water	53
Bz	—	—	—	—	Bz	182	+ 15.9	Pyridine	44, 45, 46
—	—	Ts	—	Ts	—	157	+ 20.0	Pyridine	111
1,6(?)-Dianisoyl						175–176	—		116
1,6(?)-Disalicyloyl						182–184	—		116
1,6(?)-Di-(acetylsalicyloyl)						135–136	—		116
Monosalicyloyl						148–149	—		116
1,6-Dibenzoyl-diacetylditosyl						108–109	+ 74.3	Chloroform	112
1,6-Dibenzoyl-ditosyl						137	+ 43.1	Chloroform	106
1,6-Dibenzoyl-tritosyl						133–134	+ 43.6	Chloroform	47
MY	*My*	MY	My	*My*	My	232–233	−104	Chloroform	71, 117
Ed	Ed	Ed	Ed	Ed	Ed	174	—		84, 118
Bd	Bd	Bd	Bd	Bd	Bd	218–219			84, 119
Tricinnamylidene						209	− 36.3	Chloroform	84*a*

TABLE III (*Continued*)

Substituent 6	5	4	3	2	1	*Melting point °C.*	[α]D	*Solvent*	*References*
Tri-(*o*-nitrobenzylidene)						222	− 62	Chloroform	75, 120
Tri-(*m*-nitrobenzylidene)						268	− 36	Chloroform	75, 88, 120
Isomer						248–249			119
Tri-(*p*-nitrobenzylidene)						296	− 54	Chloroform	120
Tri-(*p*-methoxybenzylidene)						235	− 21	Chloroform	120
Tri-(*p*-methylbenzylidene)						255	− 6.5	Chloroform	120
Tri-(*o*-chlorobenzylidene)						260	+ 36	Chloroform	120
Tri-(*m*-chlorobenzylidene)						212	− 23	Chloroform	120
Tri-(*p*-chlorobenzylidene)						187	+ 7	Chloroform	120
Tri-(*p*-chloro-*m*-nitro-benzylidene)						300–302	—		120
Fd	Fd	Fd	Fd	Fd	Fd	176	− 32.3	Chloroform	77
ID	ID	*Id*	*Id*	Id	Id	68–70	+ 12.5	Abs. alc.	121, 122
—	*My*	My	*My*	My	—	139	+ 71.1	Water	82, 123
1,3:4,6- or 1,3:5,6-Di-methylene						204–208	− 91.0	Water	123
Di-(chloroethylidene)						135	—		118
Di-(bromoethylidene)						137–141	—		124
—	Bd	Bd	Bd	Bd	—	203–205	+ 76.7	Pyridine	105
Di-(*p*-nitrobenzylidene)						315–320	+106	Pyridine	120
Isomer						285	+ 57	Pyridine	120
—	—	*Id*	*Id*	Id	Id	37–39	+ 19.3	Water	122, 125
Id	Id	—	—	Id	Id	122	+ 1.2	Water	53, 126, 127
—	My	—	—	My	—	173–174	− 51.4	Water	117
—	—	Ed	Ed	—	—	107–109	+ 37.7	Water	128
—	—	Bd	Bd	—	—	136–137	+ 29.0	Water	105, 106
Mono-(*p*-nitrobenzylidene)						198	—		89
Monofurfurylidene						126	+ 19.0	Water	77
—	—	Id	Id	—	—	84–85	+ 30.4	Water	109, 125, 129
—	—	—	—	Id	Id	167	+ 3.5	Water	53
Me	Me	Me	Me	Me	Me	Sirup	+ 12.5	No solvent	50
Hexaallyl						Sirup	+ 14.0	Ethanol	90
Me	Me	Me	Me	Me	—	Sirup	+ 8.9	Ethanol	49
Me	—	Me	Me	Me	—	Sirup	+ 20.7	Ethanol	50
							+ 22.1	Water	49, 125
Me	Me	—	—	Me	Me	Sirup	− 13.0	Water	49, 125
Me	—	—	—	—	Me	127–129	+ 17.0	Chloroform	128
Me	Me	—	—	—	—	93	− 7.4	Water	125
3,4-Diallyl						111–112	+ 44.7	Water	129*a*
Tr	—	—	—	—	Tr	98–103	− 3.5	Benzene	51, 108
—	—	Me	—	—	—	133–134	+ 16.7	Water	130
3-Allyl						119–120	+ 15.8	Water	129*a*
Ac	*My*	My	*My*	My	Ac	105–106	+ 98.3	Chloroform	123
Bz	*My*	My	*My*	My	Bz	120–122	+ 47.5	Chloroform	82, 123
Ts	*My*	My	*My*	My	Ts	164–165	+ 68.1	Chloroform	82, 123
1,6-Dimethacrylyl-2,4:3,5-dimethylene						85	+ 88.7	Chloroform	84*a*

TABLE III (*Continued*)

Substituent						*Melting point, °C.*	$[\alpha]_D$	*Solvent*	*References*
6	*5*	*4*	*3*	*2*	*1*				
1,6-Diacrylyl-2,4:3,5-dimethylene						86	+ 89.8	Chloroform	84*a*
Me	*My*	My	*My*	My	Me	65	+ 74.9	Chloroform	82, 128
Tr	*My*	My	*My*	My	Tr	210	+ 24.0	Chloroform	82
Ac	Bd	Bd	Bd	Bd	Ac	185–186	+100	Chloroform	105
Bz	Bd	Bd	Bd	Bd	Bz	169–170	+ 45.2	Chloroform	105
Dimorph						179–180			105
Ts	Bd	Bd	Bd	Bd	Ts	185–186	+ 87.5	Chloroform	105
Ts	Ac	*Id*	*Id*	Id	Id	106	+ 15.4	Chloroform	99
Id	*Id*	Ac	Ac	Id	Id	123	+ 26.7	Chloroform	53
Id	*Id*	Ts	Ts	Id	Id	120–121	+ 9.3	Chloroform	109
3,4-Diallyl-1,2:5,6-Diisopropylidene						Sirup			129*a*
Diacetyl-dimethylene						166	− 64.4	Chloroform	123
Dibenzoyl-dimethylene						180	+ 9.5	Chloroform	123
Ditosyl-dimethylene						147	− 37.3	Chloroform	123
Me	Me	*Id*	*Id*	Id	Id	Sirup	+ 25.7	Water	125
4-Methyl-diisopropylidene						57–58	+ 9.0	Ethanol	130
Bz	My	Bd	Bd	My	Bz	151–152	+ 61.2	Chloroform	117
Ac	My	Ac	Ac	My	Ac	117–118	− 1.3	Chloroform	117
Bz	My	Bz	Bz	My	Bz	107–108	− 7.5	Chloroform	117
Ts	My	Ts	Ts	My	Ts	177–178	+ 3.5	Chloroform	117
Ts	My	Ac	Ac	My	Ac	105–106	+ 20.7	Chloroform	131
Bz	My	—	—	My	Bz	119–120	− 70.3	Chloroform	117
Ts	My	—	—	My	Ts	148–149	− 22.6	Acetone	131
1,6-Dibenzoyl-monomethylene						154	+ 25.0	Chloroform	82
—	My	—	—	My	Ts	124–125	− 32.6	Acetone	131
Me	—	Ed	Ed	—	Me	59–60	+ 33.5	Chloroform	128
Bz	Bz	Bd	Bd	Bz	Bz	127–128	− 27.9	Chloroform	48, 105
Bz	—	Bd	Bd	Bz	Bz	140	+ 11.8	Pyridine	48
Bz	—	Bd	Bd	—	Bz	119–120	+ 31.8	Chloroform	48, 105, 106
Ac	Ac	Ac	Ac	Id	Id	107	+ 28.0	Chloroform	53
Bz	Bz	Bz	Bz	Id	Id	114	+ 47.8	Chloroform	53
Bz	Bz	Id	Id	Bz	Bz	122–123	+ 15.4	Toluene	48, 115
Bz	Ac	Id	Id	Ac	Bz	75	+ 21.2	Chloroform	46, 48, 106
Bz	Ts	Id	Id	Ts	Bz	96–97	+ 27.0	Chloroform	109
Tr	Ac	Id	Id	Ac	Tr	143	*ca.* 0		47
Me	Me	Id	Id	Me	Me	Sirup	+ 39.1	Water	125
Dibenzoyl-monotosyl-monoisopropylidene						130	+ 40.0	Chloroform	106
Ts	Ac	Id	Id	Ac	Ts	111–113	+ 25.2	Chloroform	128
Bz	—	Id	Id	—	Bz	96.5	+ 41.4	Acetone	47, 106
Me	—	Id	Id	—	Me	Sirup	+ 26.0	Chloroform	128
Tr	—	—	—	Id	Id	45–55	+ 3.3	Chloroform	53
Ac	Ac	Ac	Ac	Ac	Tr	163–164	+ 35.5	Chloroform	132
Ac	Ac	Me	Ac	Ac	Ac	85–86	+ 35.4	Chloroform	130
Tr	Ac	Ac	Ac	Ac	Tr	180–181	+ 46.4	Chloroform	108, 113
Tr	Bz	Bz	Bz	Bz	Tr	185	+ 46.9	Chloroform	108, 114

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Table IV

Derivatives of Dulcitol[133]

$$
\begin{array}{cccccccccccc}
 & & & & \mathrm{H} & & \mathrm{OH} & & \mathrm{OH} & & \mathrm{H} & \\
(\mathrm{CH_2OH} & — & & & \mathrm{C} & — & \mathrm{C} & — & \mathrm{C} & — & \mathrm{C} & —\mathrm{CH_2OH}) \\
 & & & & \mathrm{OH} & & \mathrm{H} & & \mathrm{H} & & \mathrm{OH} & \\
(6) & & & & (5) & & (4) & & (3) & & (2) & (1)
\end{array}
$$

(Abbreviations are defined on page 229)

Substituent 6	5	4	3	2	1	Melting point, °C.	References
Ac	Ac	Ac	Ac	Ac	Ac	168–169	134, 135
Bz	Bz	Bz	Bz	Bz	Bz	189–191	134
Hexa-(*p*-chlorobenzoyl)						238	101
Hexaphenylcarbamate						315 *d.*	104*a*
Ac	Bz	Ac	Ac	Bz	Ac	157–158	136
Bz	Ac	Ac	Ac	Ac	Bz	225–226	137
Ac	—	—	—	—	Ac	167–168	137
Bz	—	—	—	—	Bz	209	126, 137
Tri-(*o*-nitrobenzylidene)						92–94	138
My	—	*My*	My	—	My	249–250	139, 140
Bd	—	*Bd*	Bd	—	Bd	215–220 d.	27, 136
1,3:4,6-Di-(*o*-nitrobenzylidene)						261–262	141, 142
—	Bd	Bd	Bd	Bd	—	149–150	143
Isomer						173–174	143
—	Id	Id	Id	Id	—	111	115, 137
Diisopropylidene						98	78
Mono-(*m*-nitrobenzylidene)						256	89
Mono-(*p*-nitrobenzylidene)						186	89
Hexaallyl						Sirup	129*a*
Tr	—	—	—	—	Tr	183–184	132
—	Be	—	—	Be	—	168–169	136
My	Ac	*My*	My	Ac	My	264–265	139, 140
My	Bz	*My*	My	Bz	My	233–234	139, 140
My	Ts	*My*	My	Ts	My	220 d.	140
My	Be	*My*	My	Be	My	164	140
Bd	Ac	*Bd*	Bd	Ac	Bd	265 d.	136
2,5-Bis-(dichloroacetyl)-1,3:4,6-dibenzylidene						228–229 d.	136
Bd	Bz	*Bd*	Bd	Bz	Bd	285 d.	136
Bd	Ts	*Bd*	Bd	Ts	Bd	215 d.	136
Bd	Be	*Bd*	Bd	Be	Bd	246–250 d.	136
1,3:4,6-Di-(*o*-nitrobenzylidene)-2,5-diacetyl						320–321	141
1,3:4,6-Di-(*o*-nitrobenzylidene)-2,5-dibenzoyl						320–321	141, 142
1,3:4,6-Di-(*o*-nitrobenzylidene)-2,5-ditosyl						221–222 d.	141
Ac	Bd	Bd	Bd	Bd	Ac	168–169	143
Isomer						167–168	143
Bz	Bd	Bd	Bd	Bd	Bz	119–120	143
Isomer						147–148	143

TABLE IV (*Continued*)

Substituent						Melting point, °C.	References
6	*5*	*4*	*3*	*2*	*1*		
Ts	Bd	Bd	Bd	Bd	Ts	167–168	143
Isomer						175–176	143
Tr	Bd	Bd	Bd	Bd	Tr	184–186	143
Isomer						240–242	143
Tr	Bd	Bd	Bd	Bd	Tr	233–234	132
Ac	Id	Id	Id	Id	Ac	134	137
Bz	Id	Id	Id	Id	Bz	183–184	137
Ts	Id	Id	Id	Id	Ts	165–166	137
Tr	Id	Id	Id	Id	Tr	233–234	137
Bd	—	Bd	*Bd*	Be	*Bd*	164–165	136
Tr	Ac	Ac	Ac	Ac	Tr	237–238	132

(133) Those compounds reported by Emil Fischer as derivatives of dulcitol (ref. 115, 116), but which may actually be derivatives of D,L-galactitol (see ref. 137), have not been included.

(134) H. Rogerson, *J. Chem. Soc.*, **101**, 1040 (1912).

(135) G. Bouchardat, *Ann. chim. phys.*, [4] **27**, 145 (1872).

(136) W. T. Haskins, R. M. Hann and C. S. Hudson, *J. Am. Chem. Soc.*, **64**, 132 (1942).

(137) R. M. Hann, W. D. Maclay and C. S. Hudson, *J. Am. Chem. Soc.*, **61**, 2432 (1939).

(138) I. Tanasescu and I. Iliescu, *Bull. soc. chim.*, [5] **5**, 1446 (1938).

(139) K. Weber and B. Tollens, *Ber.*, **30**, 2510 (1897), *Ann.*, **299**, 316 (1898).

(140) R. M. Hann, W. T. Haskins and C. S. Hudson, *J. Am. Chem. Soc.*, **64**, 986 (1942).

(141) R. M. Hann, W. T. Haskins and C. S. Hudson, *J. Am. Chem. Soc.*, **64**, 1614 (1942).

(142) I. Tanasescu and E. Macovski, *Bull. soc. chim.*, [4] **53**, 1097 (1933).

(143) W. T. Haskins, R. M. Hann and C. S. Hudson, *J. Am. Chem. Soc.*, **64**, 136, 137 (1942).

TABLE V

Derivatives of D-*Galactitol*

$$\begin{array}{ccccccccccc} & & \mathrm{H} & & \mathrm{OH} & & \mathrm{OH} & & \mathrm{H} & & \\ (\mathrm{CH_2OH} & - & \mathrm{C} & - & \mathrm{C} & - & \mathrm{C} & - & \mathrm{C} & - & \mathrm{CH_2OH}) \\ & & \mathrm{OH} & & \mathrm{H} & & \mathrm{H} & & \mathrm{OH} & & \\ (6) & & (5) & & (4) & & (3) & & (2) & & (1) \end{array}$$

(Abbreviations are defined on page 229)

Substituent						*Melting point, °C.*	$[\alpha]_D$	*Solvent*	*References*
6	*5*	*4*	*3*	*2*	*1*				
Me	Me	—	Me	Me	Me	23.5	−17.1	Abs. alc.	144
Me	Me	—	Me	Me	—	83–84	−26.8	Water	144
Me	Me	Bz	Me	Me	Me	Sirup	± 0.5	Acetone	144
Me	Me	Bz	Me	Me	Bz	Sirup	−22.1	Acetone	144
Me	Me	Bz	Me	Me	—	Sirup	− 1.2	Acetone	144

TABLE VI

Derivatives of L-*Iditol*

$$\begin{array}{ccccccccccc} & & \mathrm{OH} & & \mathrm{H} & & \mathrm{OH} & & \mathrm{H} & & \\ (\mathrm{CH_2OH} & - & \mathrm{C} & - & \mathrm{C} & - & \mathrm{C} & - & \mathrm{C} & - & \mathrm{CH_2OH}) \\ & & \mathrm{H} & & \mathrm{OH} & & \mathrm{H} & & \mathrm{OH} & & \\ (6) & & (5) & & (4) & & (3) & & (2) & & (1) \end{array}$$

(Abbreviations are defined on page 229)

Substituent						*Melting point, °C.*	$[\alpha]_D$	*Solvent*	*References*
6	*5*	*4*	*3*	*2*	*1*				
Ac	Ac	Ac	Ac	Ac	Ac	121–122	−25.5	Chloroform	17, 145
Bd	Bd	Bd	Bd	Bd	Bd	224–228	—		18
Bd	Bd	Bd	Bd	Bd	Bd	*ca.* 249	—		37
—	*My*	My	*My*	My	—	260–262 d.	+39.2	Water	145
Dibenzylidene						190	—		37
Hexaallyl						Sirup	+ 3.0	Abs. alc.	129*a*
Ac	*My*	My	*My*	My	Ac	219–220	− 3.9	Chloroform	145
Bz	*My*	My	*My*	My	Bz	242–243	+38.6	Chloroform	145
Ts	*My*	My	*My*	My	Ts	187–188	+ 9.0	Chloroform	145

(144) R. S. Tipson and P. A. Levene, *J. Biol. Chem.*, **129,** 575 (1939).
(145) R. M. Hann and C. S. Hudson, *J. Am. Chem. Soc.*, **67,** 602 (1945).

TABLE VII

Derivatives of L*-Glucitol*

$$\begin{array}{ccccccccccc} & & \mathrm{OH} & & \mathrm{OH} & & \mathrm{H} & & \mathrm{OH} & & \\ (\mathrm{CH_2OH} & — & \mathrm{C} & — & \mathrm{C} & — & \mathrm{C} & — & \mathrm{C} & — & \mathrm{CH_2OH}) \\ & & \mathrm{H} & & \mathrm{H} & & \mathrm{OH} & & \mathrm{H} & & \\ (6) & & (5) & & (4) & & (3) & & (2) & & (1) \end{array}$$

(Abbreviations are defined on page 229)

Substituent						*Melting point, °C.*	$[\alpha]_D$	*Solvent*	*References*
6	*5*	*4*	*3*	*2*	*1*				
Ac	Ac	Ac	Ac	Ac	Ac	98–99	−10	Chloroform	11*d*
My	My	My	My	My	My	203	+30	Chloroform (?)	20
Dibenzylidene						160	−28	Chloroform (?)	20

TABLE VIII

Derivatives of L*-Mannitol*

$$\begin{array}{ccccccccccc} & & \mathrm{OH} & & \mathrm{OH} & & \mathrm{H} & & \mathrm{H} & & \\ (\mathrm{CH_2OH} & — & \mathrm{C} & — & \mathrm{C} & — & \mathrm{C} & — & \mathrm{C} & — & \mathrm{CH_2OH}) \\ & & \mathrm{H} & & \mathrm{H} & & \mathrm{OH} & & \mathrm{OH} & & \\ (6) & & (5) & & (4) & & (3) & & (2) & & (1) \end{array}$$

(Abbreviations are defined on page 229)

Substituent						*Melting point, °C.*	$[\alpha]_D$	*Solvent*	*References*
6	*5*	*4*	*3*	*2*	*1*				
Ac	Ac	Ac	Ac	Ac	Ac	122–123	− 25.2	Chloroform	26
My	My	My	My	My	My	227	+106	Chloroform	26
Id	Id	Id	Id	Id	Id	69–70	− 12.6	Abs. alc.	26
Id	Id	—	—	Id	Id	122	—		26, 127
—	—	Id	Id	—	—	85–86.5	− 29.6	Water	146
Me	Me	—	—	Me	Me	Sirup	+ 13.2	Water	146
Me	Me	Id	Id	Me	Me	Sirup	− 39.0	Water	146

TABLE IX

Derivative of D*-Iditol*

$$\begin{array}{ccccccccccc} & & \mathrm{H} & & \mathrm{OH} & & \mathrm{H} & & \mathrm{OH} & & \\ (\mathrm{CH_2OH} & — & \mathrm{C} & — & \mathrm{C} & — & \mathrm{C} & — & \mathrm{C} & — & \mathrm{CH_2OH}) \\ & & \mathrm{OH} & & \mathrm{H} & & \mathrm{OH} & & \mathrm{H} & & \\ (6) & & (5) & & (4) & & (3) & & (2) & & (1) \end{array}$$

(Abbreviations are defined on page 229)

Substituent						*Melting point, °C.*	$[\alpha]_D$	*Solvent*	*References*
6	*5*	*4*	*3*	*2*	*1*				
Ac	Ac	Ac	Ac	Ac	Ac	121–122	+25.3	Chloroform	19, 147

(146) E. Baer and H. O. L. Fischer, *J. Biol. Chem.*, **145**, 61 (1942).
(147) R. M. Hann and C. S. Hudson, *J. Am. Chem. Soc.*, **64**, 925 (1942).

TABLE X

Derivatives of D-*Talitol*

$$\begin{array}{c} \mathrm{H}\quad\mathrm{OH}\quad\mathrm{OH}\quad\mathrm{OH} \\ (\mathrm{CH_2OH—C—C—C—C—CH_2OH}) \\ \mathrm{OH}\quad\mathrm{H}\quad\mathrm{H}\quad\mathrm{H} \\ (6)\quad(5)\quad(4)\quad(3)\quad(2)\quad(1) \end{array}$$

(Abbreviations are defined on page 229)

Substituent						*Melting point, °C.*	$[\alpha]_D$	*Solvent*	*References*
6	*5*	*4*	*3*	*2*	*1*				
MY	MY	*My*	My	*My*	My	118–119	−32.1	Water	29
Bd	Bd	Bd	Bd	Bd	Bd	210	−40	Chloroform	27, 148
My	—	*My*	My	—	My	182–183	−41.2	Water	29
—	My	My	My	My	—	261–262	− 1.0	Water	29
—	—	My	—	My	—	144–145	− 4.2	Water	29
Hexaallyl						Sirup	—		129*a*
My	Ts	*My*	My	Ts	My	193–194	−27.9	Chloroform	29
My	Ac	*My*	My	Ac	My	190–191	−27.5	Chloroform	29
Ts	My	My	My	My	Ts	204–205	+ 2.8	Acetonyl acetone	29
Ac	My	My	My	My	Ac	177–178	− 0.5	Chloroform	29
Bz	My	My	My	My	Bz	188–189	+ 1.0	Chloroform	29
Ac	Ac	My	Ac	My	Ac	67–68	+37.8	Chloroform	29
Bz	Bz	My	Bz	My	Bz	122–123	+ 8.1	Chloroform	29

TABLE XI

Derivatives of Allitol

$$\begin{array}{c} \mathrm{H}\quad\mathrm{H}\quad\mathrm{H}\quad\mathrm{H} \\ (\mathrm{CH_2—C—C—C—C—CH_2OH}) \\ \mathrm{OH}\quad\mathrm{OH}\quad\mathrm{OH}\quad\mathrm{OH} \\ (6)\quad(5)\quad(4)\quad(3)\quad(2)\quad(1) \end{array}$$

(Abbreviations are defined on page 229)

Substituent						*Melting point, °C.*	*References*
6	*5*	*4*	*3*	*2*	*1*		
Ac	Ac	Ac	Ac	Ac	Ac	61	32
Dibenzylidene						249–250	149
—	*My*	My	*My*	My	—	256–257	11*c*
Ac	*My*	My	*My*	My	Ac	176–177	11*c*
Ts	*My*	My	*My*	My	Ts	200–202	11*c*
2,4:3,5-Dimethylene-1,6-dilauroyl						108–109	11*c*

(148) C. A. Lobry de Bruyn and W. Alberda van Ekenstein, *Rec. trav. chim.*, **18,** 150 (1899).

(149) R. Lespieau and J. Wiemann, *Bull. soc. chim.*, [4] **53,** 1107 (1933).

PLANT GUMS AND MUCILAGES

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Contents

I. Plant Gums

1. *Composition*

Plant gums may be defined as those substances of plant origin which are obtained as exudations from the fruit, trunks or branches of trees spontaneously or after mechanical injury of the plant by incision of the bark or by removal of a branch, or after invasion by bacteria or fungi.

It has also been suggested that gums result from normal plant metabolism. The gums appear to be produced by some protective mechanism in order to seal off the injured portion from attack by organisms. The origin of the gum is obscure but some authorities consider that it arises from starch granules present in the cells. These granules disappear and it has been suggested that they are converted into the gum which is exuded. Whether the injury to the tree causes it to synthesize the gum from materials already present in the sap or whether the gum is produced by the invading organisms is still a matter for speculation at the present

time. It is, however, of interest to note that partly hydrolyzed plant gums are not only structurally related to some pneumococcus polysaccharides but in addition they give a precipitin reaction with antipneumococcus sera.[1] This might be taken as an indication that the injured trees produce gums as a protection against further attack by organisms.

One remarkable feature of plant gums is the striking uniformity in the structure of gums isolated from different trees of the same type. For example, damson plum trees produce a gum whose properties do not vary from tree to tree.[2] The same has been said by O'Sullivan about gum arabic from acacia trees.[3,4]

The plant gums are amorphous substances containing carbon, hydrogen and oxygen and they are members of the carbohydrate group. In many cases small amounts of nitrogen are detectable,[5] this may be traceable to the proteinaceous debris arising from the enzyme which is responsible for the formation of the gums or it may arise from contact of the gum with protein material of the tree. The plant polysaccharide gums are hydrophilic substances and are characterized by dissolving in cold water or taking up water to form a mucilage.

An attempt has been made to classify gums into two main groups: (a) real gums which are those plant products which form a clear solution in water and (b) vegetable mucilages which are those which swell but do not dissolve completely in water.[6] This classification is useful but not entirely satisfactory since there are exceptions. Thus gum tragacanth, a tree exudate and a true plant gum, is only partially soluble in water[5,7] and exhibits those properties normally attributed to mucilages. In this section of the article the term plant gum will be restricted to those complex acid polysaccharides which are exuded from trees either spontaneously or after mechanical injury.

The plant gums are neutral salts of complex polysaccharide acids, composed of hexose residues, uronic acid residues, pentose residues, and methylpentose residues, which are joined together in the most diverse manner within the same molecule. With the exception of gum tragacanth, which contains D-galacturonic acid units, the plant gums are distinguished by the fact that D-glucuronic acid is the acid component present in them all.

(1) M. Heidelberger, O. T. Avery and W. F. Goebel, *J. Exp. Med.*, **49**, 847 (1929).
(2) E. L. Hirst and J. K. N. Jones, *J. Chem. Soc.*, 1174 (1938).
(3) C. O'Sullivan, *J. Chem. Soc.*, **59**, 1029 (1891).
(4) C. Scheibler, *Ber.*, **6**, 612 (1873).
(5) C. O'Sullivan, *J. Chem. Soc.*, **79**, 1164 (1901).
(6) Morris B. Jacobs, "The Chemistry and Technology of Food and Food Products," Interscience, New York, Vol. I, p. 819 (1944).
(7) Sybil P. James and F. Smith, *J. Chem. Soc.*, 739 (1945).

Closely related to plant gums are those mucilages discussed later which are the complex acid polysaccharides extractable from endosperms of seeds of such plants as flax, quince and lucerne (see below). The acid nature of these mucilages is due to the presence of D-galacturonic acid residues in the complex molecule.[8–10]

The present state of our knowledge of plant gums indicates that the uronic acid component (D-glucuronic acid) is present in the pyranose form. The hexoses encountered in plant gums are D-galactose and D-mannose and they too have the pyranose form. Glucose has not been found in any of the plant gums examined so far. The pentose arabinose is always found in the furanose form and is a member of the L-series of sugars while xylose, which occurs in the pyranose modification, belongs to the D-series. The methylpentoses found in plant gums are L-rhamnose and L-fucose and these assume the pyranose structure. The significance of the occurrence of so many sugars united by all the known types of glycosidic linkage is not known unless it be that they are so constituted as to be able to resist the action of an organism or series of invading organisms with their accompanying enzyme systems.

It will be apparent that the determination of the structure of plant gums is dependent upon the methods which have been elaborated for the elucidation of the structure of the simple sugars and for the synthesis of sugar derivatives of known structure. Upon the basis of this knowledge of the structure and the fundamental reactions of the monosaccharides and disaccharides these complex plant gum polysaccharides have been examined with some success. The work of early investigators on the simple sugars has proved invaluable in these studies on plant gums and the authors would also pay tribute to those workers in this difficult field who made progress at the time when there was little knowledge of the chemistry of even simple monosaccharides.

The determination of the structure of a plant gum involves the establishment of its homogeneity, its equivalent weight, and rotation and its uronic acid and pentosan content by the well-known methods. The nature of the constituent sugars and the uronic acid are then determined, after hydrolysis of the gums, by crystallization or by conversion into characteristic crystalline derivatives. As an example the hexose mannose may be identified as the free sugar or in the form of its anilide, methyl glycoside or phenylhydrazone. Autohydrolysis of the acid

(8) R. E. Gill, E. L. Hirst and J. K. N. Jones, *J. Chem. Soc.*, 1469 (1939).

(9) R. S. Tipson, C. C. Christman and P. A. Levene, *J. Biol. Chem.*, **128**, 609 (1939).

(10) E. Anderson and M. Fireman, *J. Biol. Chem.*, **109**, 437 (1935); E. Anderson, L. A. Gillette and M. G. Seeley, *ibid.*, **140**, 569 (1941).

polysaccharide or hydrolysis with dilute mineral acid can sometimes be effected in such a way as to remove the pentofuranose units[3,11] together with any other sugars which may be attached to them,[12] leaving a nucleus or skeleton of the gum which is composed only of residues of the pyranose type. This has proved highly effective in several cases since the structures of the degraded products are much less complex and more easily determined. Further hydrolysis of this nucleus of the degraded gum often results in the production of a hexose such as galactose and an aldobionic acid, which consists of glucuronic acid linked to another hexose such as galactose or mannose. By these means the constituents of the gum may be determined and an idea of their mode of union may be deduced. The complex nature of the plant gums may be seen by an examination of Table I in which are given the products of hydrolysis of several gums.[13]

TABLE I

Composition of Plant Gums[13]

Gum	D-*Glucuronic acid*	D-*Galacturonic acid*	D-*Galactose*	D-*Mannose*	L-*Rhamnose*	L-*Fucose*	L-*Arabinose*	D-*Xylose*
Almond	+	−	+	−	−	−	+	+
Arabic	+	−	+	−	+	−	+	−
Cherry	+	−	+	+	−	−	+	+
Cholla	+	−	+	−	?+	−	+	+
Damson	+	−	+	+	−	−	+	+
Egg plum	+	−	+	−	−	−	+	+
Grapefruit	+	−	+	−	−	−	+	−
Lemon	+	−	+	−	−	−	+	−
Mesquite	+	−	+	−	−	−	+	−
Orange	+	−	+	−	−	−	+	−
Purple plum	+	−	+	−	−	−	+	+
Tragacanth	−	+	+	−	−	+	+[a]	+

[a] Gum tragacanth is a mixture consisting of a salt of a complex acidic polysaccharide (tragacanthic acid) and a neutral polysaccharide which contains arabinose units. It is the latter which probably accounts for the isolation of arabinose from the crude gum.[7]

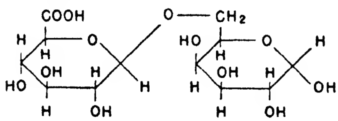
Preliminary investigations into the constitution of plant gums have been directed to the isolation of disaccharides or aldobionic acids which are relatively easy to identify. Thus the structures of the aldobionic

(11) E. Anderson and Lila Sands, *J. Am. Chem. Soc.*, **48**, 3172 (1926); E. V. White, *ibid.*, **69**, 715 (1947).

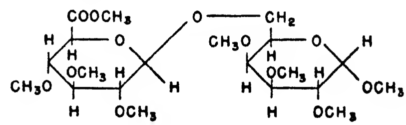
(12) F. Smith, *J. Chem. Soc.*, 744 (1939).

(13) E. L. Hirst, *J. Chem. Soc.*, 70 (1942).

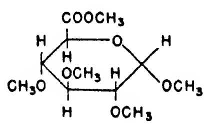
acids derived from gums by hydrolysis have been established chiefly by methods used in studies on the reducing disaccharides. For instance, the aldobionic acid I obtained from gum arabic by acid hydrolysis undergoes methylation to give the methyl ether II which upon methanolysis affords the methyl ester of methyl 2,3,4-trimethyl-D-glucuronoside (III) and methyl 2,3,4-trimethyl-D-galactoside (IV). These cleavage fragments have been identified by their conversion into characteristic crystalline derivatives.[14,15] The aldobionic acid I has also been obtained from egg plum gum[16] and from mesquite gum (as a methyl derivative).[17]



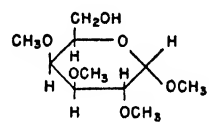
I



II

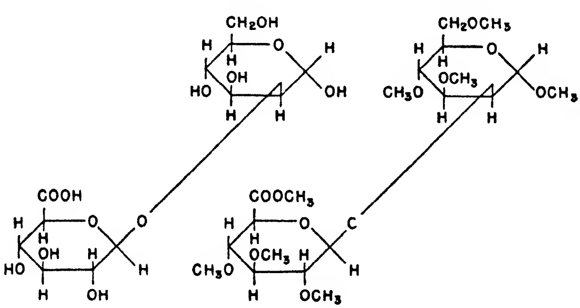


III

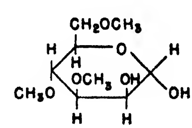


IV

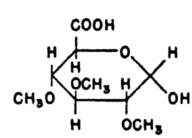
The same procedure has revealed that the aldobionic acid obtained from damson gum and cherry gum by acid hydrolysis has the structure V.[2,18] Methylation gives the methyl ether VI and when this is subjected to hydrolysis with dilute mineral acid there results 3,4,6-trimethyl-D-mannose (VII) and 2,3,4-trimethyl-D-glucuronic acid (VIII).



V VI



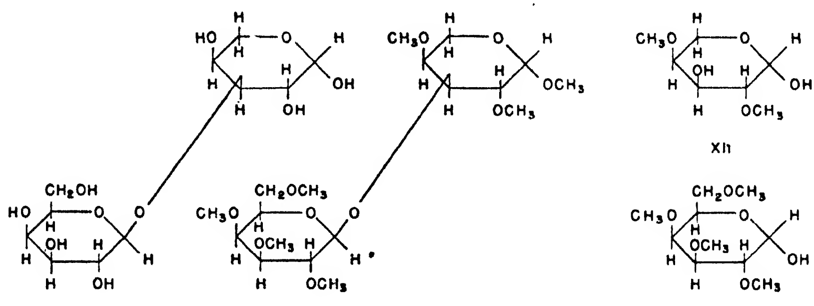
VII



VIII

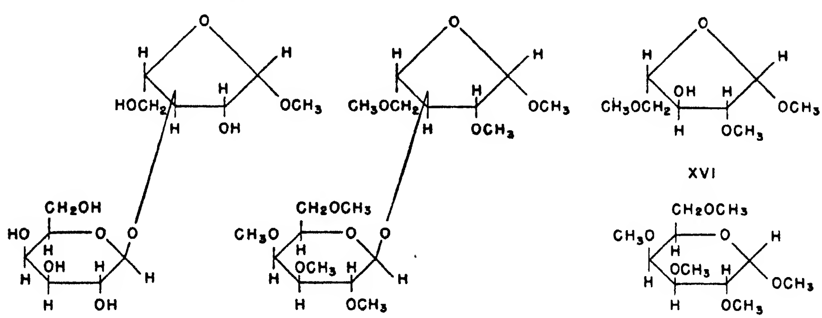
(14) S. W. Challinor, W. N. Haworth and E. L. Hirst, *J. Chem. Soc.*, 258 (1931).
(15) J. Jackson and F. Smith, *J. Chem. Soc.*, 74 (1940).
(16) E. L. Hirst and J. K. N. Jones, *J. Chem. Soc.*, 1064 (1947); 120 (1948).
(17) J. I. Cunneen and F. Smith, *J. Chem. Soc.*, 1141 (1948).
(18) J. K. N. Jones, *J. Chem. Soc.*, 558 (1939).

The novel type of disaccharide IX, consisting of a unit of D-galactose joined to a unit of L-arabinose, was obtained by autohydrolysis of gum arabic. The structure of this disaccharide was proved by the fact that methylation yielded the heptamethyl ether X and this gave on hydrolysis 2,3,4,6-tetramethyl-D-galactose (XI) and 2,4-dimethyl-L-arabinose (XII).



IX X XI

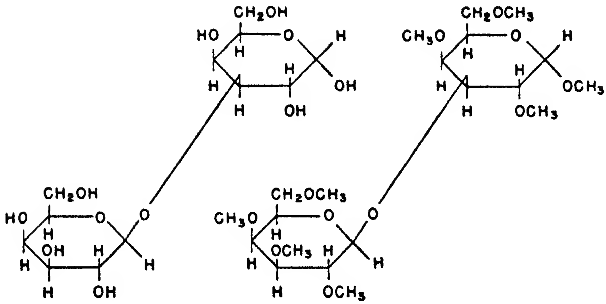
Preliminary treatment of the disaccharide IX with methyl alcoholic hydrogen chloride at room temperature, followed by methylation and subsequent methanolysis, enabled the series of reactions XIII → XIV → XV + XVI to be effected. Identification of the two fragments XV and XVI was then carried out in the usual way.[12]



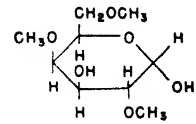
XIII XIV XV

More drastic hydrolysis of gum arabic has led to the isolation of 3-D-galactopyranosyl-D-galactose (XVII), the structure of which was ascertained by the fact that treatment of it with methyl sulfate gave the octamethyl derivative (XVIII), which upon hydrolysis afforded 2,3,4,6-tetramethyl-D-galactose (XI) and 2,4,6-trimethyl-D-galactose (XIX).[19]

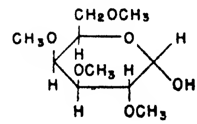
(19) J. Jackson and F. Smith, *J. Chem. Soc.*, 79 (1940).



XVII XVIII



XIX



XI

These preliminary investigations gave an idea of the main features of plant gums and it became obvious that gums are not only complex in the sense that they are composed of different sugar units but that these units are joined by more than one type of glycosidic linkage. With the exception of the polysaccharide xylan,[20] the complexity encountered here, arising from the presence of different sugars in the same molecular complex, had not been recognized and it is only relatively recently that the formation of a preponderance of 2,3-dimethyl-D-glucose in the dimethyl fraction from methyl starch and methyl glycogen has been taken to indicate that its origin is due to the existence of 1,6 and 1,4 linkages and not to incomplete methylation.[21–23]

It was apparent that solution of the problem of the complexity of plant gums due to the different sugars and linkages could only be obtained by studies on the gum itself and the degraded nuclear portion of the complex to which the labile pentose units were originally attached, as well as an examination of smaller degradation products which could be isolated. The plant gums which have been subjected to recent and extensive study along these lines and to which reference will now be made are gum arabic, damson gum, cherry gum, mesquite gum and gum tragacanth. These investigations have progressed to some extent and it is possible to give a rough picture of the structure of several plant gums though it must be pointed out that, for the time being, it is not yet possible to assign a particular formula to any one gum.

(20) W. N. Haworth, E. L. Hirst and Elsie Oliver, *J. Chem. Soc.*, 1917 (1934).
(21) C. C. Barker, E. L. Hirst and G. T. Young, *Nature*, **147**, 296 (1941).
(22) K. Freudenberg and H. Boppel, *Naturwissenschaften*, **28**, 264 (1940); *Ber.*, **63**, 609 (1940).
(23) K. Myrbäck and K. Ahlborg, *Biochem. Z.*, **307**, 49 (1940).

2. *Gum Arabic*

Gum arabic was examined by Neubauer who recognised its carbohydrate character as well as the fact that it was an acid polysaccharide.[24] Gums had, however, been known to belong to the same class of organic substances as sugar and starch as long ago as 1810 as a result of the studies of Gay-Lussac and Thenard.[25] The sugar units of which gum arabic is composed are D-galactose, L-arabinose, L-rhamnose and D-glucuronic acid. Arabinose was recognised as one of the hydrolysis products by Scheibler[4] and by O'Sullivan.[26] Galactose was identified by Kiliani,[27] Claesson,[28] O'Sullivan[26] and also by Butler and Cretcher.[29] The presence of L-rhamnose was indicated[30] and later proved by the isolation of the sugar in the crystalline state[31] and in the form of crystalline derivatives.[12] Autohydrolysis of the arabic acid, a process which simply involves heating an aqueous solution of the gum acid on the water-bath, affords L-arabinose, L-rhamnose, and the 3-D-galactopyranosyl-L-arabinose (IX) to which reference has already been made.[12] Separation of these labile residues left a more resistant nucleus, degraded arabic acid, which upon prolonged hydrolysis with dilute mineral acid gives the aldobionic acid I, designated 6-β-D-glucuronosyl-D-galactose.[14,32] Autohydrolysis of the degraded arabic acid afforded the reducing D-galactose disaccharide XVII mentioned above which was shown to have a 1,3 glycosidic link.[19]

Degraded arabic acid was then methylated and the methyl derivative subjected to hydrolysis with methyl alcoholic hydrogen chloride. Examination of the cleavage fragments showed them to consist of the glycosides of the substances given in column one of Table II.

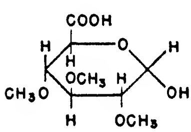
On the assumption that methylation of the degraded arabic acid was complete, the location of hydroxyl groups in the various cleavage fragments indicates the positions through which the monosaccharide units are involved in union with the other residues. Thus the isolation of three molecular proportions of 2,3,4-trimethyl-D-glucuronic acid (VIII) and one molecular proportion of 2,3,4,6-tetramethyl-D-galactose (XI)

(24) C. Neubauer, *J. prakt. Chem.*, **62**, 193 (1854); *Ann.* **102**, 105 (1857).
(25) L. J. Gay-Lussac and L. J. Thenard, *Ann. chim. phys.*, **74**, 47 (1810).
(26) C. O'Sullivan, *J. Chem. Soc.*, **45**, 41 (1884).
(27) H. Kiliani, *Ber.*, **13**, 2304 (1880).
(28) P. Claesson, *Ber.*, **14**, 1270 (1881).
(29) C. L. Butler and L. H. Cretcher, *J. Am. Chem. Soc.*, **51**, 1519 (1929).
(30) A. G. Norman, *Biochem. J.*, **23**, 524 (1929).
(31) F. Smith, *J. Chem. Soc.*, 1035 (1940).
(32) R. D. Hotchkiss and W. F. Goebel, *J. Am. Chem. Soc.*, **58**, 858 (1936); *J. Biol. Chem.*, **115**, 285 (1936).

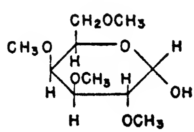
demonstrates that these residues constitute the four "end" groups in the repeating unit of the molecular complex. Similarly the isolation of 2,3,4-trimethyl-D-galactose (XX) shows that this substance arises from a D-galactose residue linked to other units in the complex through positions 1 and 6. Those D-galactose residues which give rise to the 2,4-dimethyl derivative (XXI) must be combined with other members through positions 1, 3 and 6.

TABLE II

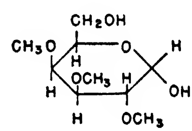
Methylated Degraded Arabic Acid	*Methylated Arabic Acid*
2,3,4,6-Tetramethyl-D-galactose (XI) (1 mole)	2,3,4,6-Tetramentyl-D-galactose (XI)
2,3,4-Trimethyl-D-galactose (XX) (5 moles)	2,3,4-Trimethyl-L-rhamnose (XXIV)
2,4-Dimethyl-D-galactose (XXI) (3 moles)	2,3,5-Trimethyl-L-arabinose (XXV)
2,3,4-Trimethyl-D-glucuronic acid (VIII) (3 moles)	2,5-Dimethyl-L-arabinose (XXVI)
	2,4-Dimethyl-D-galactose (XXI)
	2,3-Dimethyl-D-glucuronic acid (XXVII)
	2,3,4-Trimethyl-D-glucuronic acid (VIII)



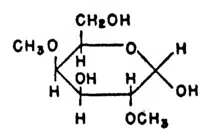
VIII



XI



XX

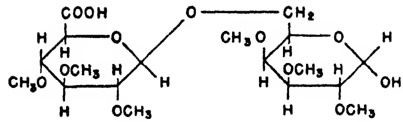


XXI

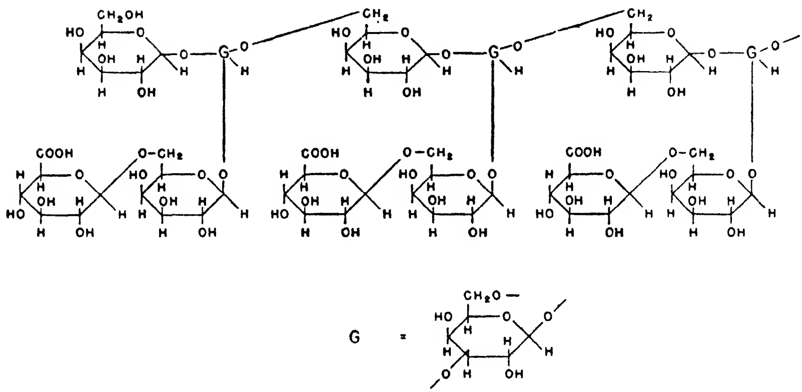
The existence of four "ends" in a molecular complex consisting of 12 members demonstrates the branched chain character of the product. Furthermore, it can be said that the branching occurs in those D-galactose units which gave rise to 2,4-dimethyl-D-galactose.

The isolation of the four products listed in Table II column 1 shows that all the residues in the stable nucleus of degraded arabic acid have the pyranose form and that all the units are joined by either 1,3 or 1,6 linkages. The possible structures which could accommodate these experimental facts were indicated[33] and later work limited the number of possibilities still further by the isolation of hexamethyl-(6-D-glucuronosyl-D-galactose) (XXII) from methylated degraded arabic acid by controlled hydrolysis.[15] One possible structure advanced for degraded arabic acid is shown in XXIII.

(33) F. Smith, *J. Chem. Soc.*, 1724 (1939).



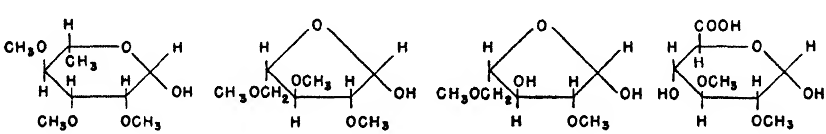
XXII



XXIII

The D-galactopyranose unit G is known to be united to other units through its 3 and 6 positions since it affords 2,4-dimethyl-D-galactose. This unit is represented, however, by the letter G in the formula because it is not yet clear whether it is joined to other galactose units in the main chain by a 1,3 linkage and to the side chains of aldobionic acid by a 1,6 linkage or *vice versa*, or whether the galactose members are joined by 1,3 and by 1,6 linkages.

The methyl derivative of gum arabic, readily formed by the action of methyl sulfate and sodium hydroxide upon the gum, gave upon hydrolysis the cleavage fragments shown in the second column of Table II.

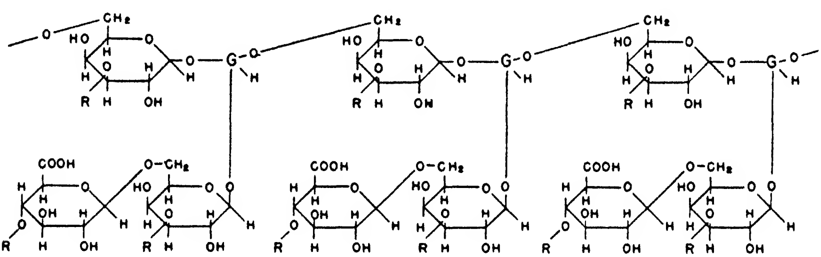


XXIV XXV XXVI XXVII

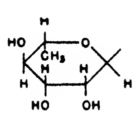
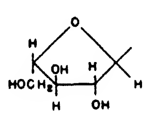
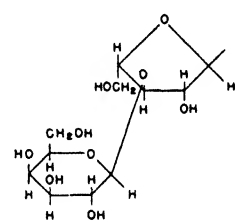
The identification of these products demonstrated that the gum molecule possesses a highly branched chain structure, a deduction already made from the study of degraded arabic acid,[33] and that those labile sugar residues, L-arabinose, L-rhamnose, and 3-D-galactopyranosyl-L-

arabinose, liberated during autohydrolysis, are joined to the nucleus of degraded arabic acid in the form of L-arabofuranose (XXIX), L-rhamnopyranose (XXVIII), and 3-D-galactopyranosyl-L-arabofuranose (XXX).[31] The residues of arabic acid which are involved in linkage with the labile sugars are clearly those D-galactose units of methylated degraded arabic acid which afford 2,3,4-trimethyl-D-galactose, and the uronic acid residue of methylated arabic acid which affords the 2,3-dimethyl-D-glucuronic acid. With the exception of that galactose residue which is obtainable as 3-D-galactopyranosyl-L-arabinose, all the galactose units are trebly linked since they afford 2,4-dimethyl-D-galactose and are involved either in union with the labile sugars or in the chain branching.

Several formulae, varying only in detail, may be assigned to gum arabic on the available experimental evidence and one of them (XXXI) is shown below.



XXXI

R =  or  or 

XXVIII XXIX XXX

3. *Damson Gum*

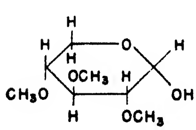
Damson gum (*Prunus insitia*) appears to be related to gum arabic in its general structure and size of repeating unit (the equivalent weights are approximately the same), but it differs from it in containing D-xylose and D-mannose but no L-rhamnose. D-Galactose, L-arabinose and D-glucuronic acid are common to both gums. As with arabic acid, damson gum undergoes ready hydrolysis with dilute mineral acid whereby the labile L-arabinose units are separated, leaving the more resistant nucleus of degraded damson gum.[2] The latter can be hydrolyzed further

to give D-galactose, a small amount of D-xylose and an aldobionic acid, 2-D-glucuronosyl-D-mannose (V). Although there are points of resemblance between gum arabic and damson gum, the identification of this aldobionic acid with a 1,2 linkage reveals one important structural difference between the two.

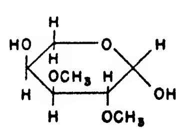
Hydrolysis of methylated degraded damson gum gave a mixture of methylated sugars even more complex than those produced from methylated degraded arabic acid. The cleavage fragments were, however, isolated, and shown by the formation of crystalline derivatives to consist of those methylated sugars listed in the first column of Table III. Some 4,6-dimethyl-D-galactose not given in Table III was also identified.[33a]

TABLE III

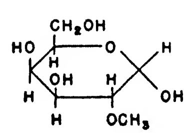
Methylated Degraded Damson Gum	*Methylated Damson Gum*
2,3,4,6-Tetramethyl-D-galactose (XI) (1 part)	2,3,5-Trimethyl-L-arabinose (XXV) (8 parts)
2,4,6-Trimethyl-D-galactose (XIX) (1 part)	2,3-Dimethyl-L-arabinose (XXXIII) (4 parts)
2,3,4-Trimethyl-D-galactose (XX) (1 part)	2,4,6-Trimethyl-D-galactose (XIX) (3 parts)
2,4-Dimethyl-D-galactose (XXI) (1 part)	2,4-Dimethyl-D-galactose (XXI) (3 parts)
2,3,4-Trimethyl-D-glucuronic acid (VIII) (1 part)	2-Methyl-D-galactose (XXXIV) (1 part)
2,3-Dimethyl-D-glucuronic acid (XXVII) (1 part)	4-Methyl-D-galactose (XXXV) (1 part)
2,3,4-Trimethyl-D-xylose (XXXII) (1/6 part)	2,3,4-Trimethyl-D-glucuronic acid (VIII) (2 parts)
	2,3-Dimethyl-D-glucuronic acid (XXVII) (2 parts)



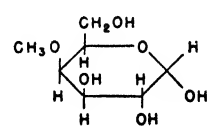
XXXII



XXXIII



XXXIV



XXXV

Methylation of the undegraded gum by the same procedure as that used for the degraded product, namely, by treatment of the thallium salt with methyl iodide (a very useful method when the methyl sulfate treatment fails[34,35a,35b]) gave the corresponding methylated gum which afforded upon hydrolysis those methylated sugars listed in the second column of

(33*a*) E. L. Hirst and J. K. N. Jones, *J. Chem. Soc.*, 1482 (1939).
(34) Christina M. Fear and A. C. Menzies, *J. Chem. Soc.*, 937 (1926).
(35*a*) E. L. Hirst and J. K. N. Jones, *J. Chem. Soc.*, 496 (1938).
(35*b*) C. S. Hudson and C. B. Purves, *J. Am. Chem. Soc.*, **59**, 49, 1170 (1937).

Table III, together with methylated derivatives of mannose and xylose not yet characterized.

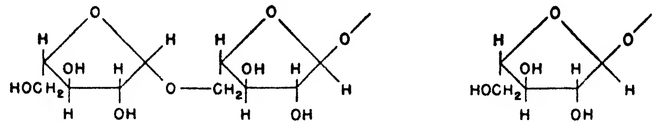
Despite the achievement of the identification of these highly complex mixtures of sugars from the methylated degraded and the methylated gum and the estimation of the quantities of each present in the mixtures, it is not possible to assign a single structural formula to this gum. Nevertheless, a comparison of the products formed from the methyl derivatives of the degraded and undegraded gum shows that 2,3,4,6-tetramethyl-D-galactose (XI) and 2,3,4-trimethyl-D-galactose (XX) do not occur among the cleavage fragments of the methylated gum (see Table III). The inference is therefore drawn that the L-arabinose units, eliminated by mild acid hydrolysis of the gum, are attached to these D-galactose molecules of degraded damson gum which give rise to the 2,3,4,6-tetramethyl-D-galactose and the 2,3,4-trimethyl-D-galactose upon hydrolytic cleavage of the methylated product. Since hydrolysis of the methylated gum gave amounts of 2,3,5-trimethyl-L-arabinose (XXV) and 2,3-dimethyl-L-arabinose (XXXIII) in the ratio of 2 to 1 it can be deduced that in the repeating unit there must be two side chains consisting of L-arabinose units, one (XXXVI*a*) composed of a single arabinose residue and the other (XXXVI) of two. These two side chains are composed of L-arabofuranose residues and it can be further stated that the two units in the one side chain are mutually joined by a 1,5 linkage as in XXXVI.

In the repeating unit of damson gum which consists of L-arabinose (3 parts), D-galactose (2 parts), D-mannose (1 part) and D-glucuronic acid (1 part), the arabinose side-chains are joined to positions 3 and 6 of the galactose residues. Since the molecular proportions of the 2,3,4-trimethyl and 2,3-dimethyl-D-glucuronic acid appear to be the same in the methylated degraded and the methylated undegraded damson gum it would seem that in the gum neither of these acid units constitutes a point of attachment of an arabinose side chain. The arabinose side chains do, however, appear to be joined to those galactose residues of the methylated gum which afford 2-methyl-D-galactose (XXXIV) and 4-methyl-D-galactose (XXXV), for these monomethyl derivatives of galactose are produced from the methylated gum and not from the methylated degraded gum. It has been pointed out that the monomethyl galactoses may arise as a result of incomplete methylation but the authors[36] prefer to believe that these monomethyl sugars have the above constitutional significance and are therefore an integral part of the methylated gum.

The results obtained from the study of damson gum have not yet enabled a formula to be assigned to this complex polysaccharide, for although residues formulated below are known to be present and their

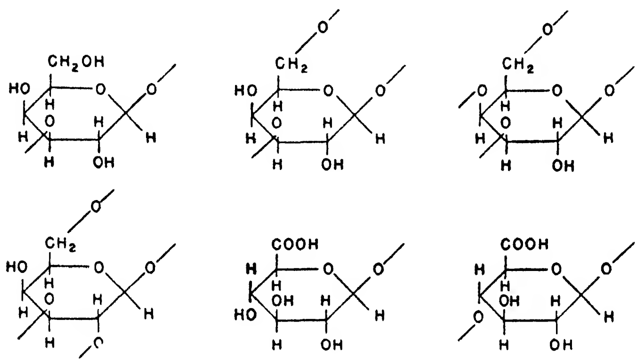
(36) E. L. Hirst and J. K. N. Jones, *J. Chem. Soc.*, 506 (1946).

mode of union (represented by free bonds in the formulas XXXVII) has been determined, the precise order of arrangement of these residues in the complex still remains to be defined.



XXXVI XXXVIa

The L-arabofuranose side chains of damson gum.



XXXVII

Residues present in degraded damson gum.

4. *Cherry Gum*

Specimens of cherry gum (*Prunus cerasus*) obtained from cultivated trees growing in England have been examined by Jones[18] and were found to be different in some respects from that studied by Butler and Cretcher[37] who obtained the gum from wild cherry trees (*Prunus virginiana* L) in the state of Indiana, U. S. A. The free acid from wild cherry tree gum had an equivalent weight of 1790 and consisted of L-arabinose (8 moles), D-xylose (6 moles), D-galactose (6 moles), D-mannose (3 moles) and D-glucuronic acid (2 moles). The acid gum from the English trees consisted of L-arabinose (6 moles), D-galactose (2 moles), D-mannose (1 mole), D-glucuronic acid (1 mole) and small amounts of D-xylose and had an equivalent weight of 1450.

Further study of the gum of the English cherry tree has shown that it bears many resemblances to damson gum. Both gums for instance upon prolonged hydrolysis give an aldobionic acid which has been proved

(37) C. L. Butler and L. H. Cretcher, *J. Am. Chem. Soc.*, **53**, 4160 (1931).

to be 2-D-glucuronosyl-D-mannose (V) by the procedure given above.[18] The fully methylated English cherry gum has been prepared by treatment of the thallium salt of the gum with methyl iodide.[35a] Cleavage of the methylated gum by methanolysis followed by fractional distillation and hydrolysis of the methyl glycosides yielded the following six methyl sugars which were recognised by their conversion into crystalline derivatives: 2,3,5-trimethyl-L-arabinose (XXV), 2,5-dimethyl-L-arabinose (XXVI), 2,4,6-trimethyl-D-galactose (XIX) 2,4-dimethyl-D-galactose (XXI), 2,3,4-trimethyl-D-glucuronic acid (VIII) and 2,3-dimethyl-D-glucuronic acid (XXVII).

A preliminary study of the unmethylated gum showed that D-mannose and small amounts of D-xylose are present in the gum[18] but methylated residues of these sugars have not yet been isolated from the methylated gum though they must be present.

Considerable difficulty was encountered in the quantitative separation and identification of the various cleavage fragments of the methylated gum and consequently it is not yet possible to assign any one formula to cherry gum. The main structural features of the gum are, however, apparent as may be seen from the following considerations. Since the two methylated arabinose derivatives XXV and XXVI formed by methanolysis have the furanose structure these L-arabinose units must be present in the gums as furanose residues, and in view of their ease of removal both from the methylated and unmethylated gum by treatment with acid, it appears that they must be joined as side chains to the acid–resistant nucleus of degraded cherry gum. The degraded cherry gum appears to resemble degraded arabic acid and degraded damson gum in that it probably consists of a main chain of D-galactose units to which are attached side chains of aldobionic acid. In both cherry gum and damson gum the D-galactose residues are linked through positions 1 and 3, and positions 1, 3 and 6, since they give rise in the case of the methylated gum to 2,4,6-trimethyl-D-galactose (XIX) and 2,4-dimethyl-D-galactose (XXI), respectively. Cherry and damson gums, like gum arabic, possess terminal units of D-glucuronic acid since the 2,3,4-trimethyl derivative (VIII) is produced as one of the cleavage fragments of the methylated gums. In addition, units of D-glucuronic acid which have a 1,4 linkage occur in each of these three gums since 2,3-dimethyl-D-glucuronic acid (XXVII) is also formed by hydrolysis of the methylated gums. One point of difference between cherry and damson gum is that cherry gum has its L-arabofuranose units mutually joined by 1,3 linkages as is the case in gum arabic since 2,5-dimethyl-L-arabinose (XXVI) is formed from the methylated gum, whereas in damson gum the L-arabofuranose residues must be joined by 1,5 glycosidic

bonds if the production of 2,3-dimethyl-L-arabinose (XXXIII) from the *methylated gum is to be explained.*[38]

5. *Egg Plum Gum*

Recent researches on a gum from the egg plum tree (*Prunus domestica*) belonging to the family *Rosaceae* have demonstrated that this gum resembles gum arabic more closely than do the damson and cherry gums inasmuch as it consists of a stable nucleus composed of D-galactose (3 moles) and D-glucuronic acid (1 mole), to which are attached L-arabinose units (3 moles). Unlike gum arabic this gum contains D-xylose (1 mole).[16] The free gum acid undergoes autohydrolysis in which process the L-arabinose and D-xylose units are eliminated and there is left the relatively stable nucleus of degraded egg plum gum which will probably be shown to be composed solely of pyranose residues analogous to the degraded residue from gum arabic. The similarity between the gum now under consideration and gum arabic is further emphasized by the fact that further acid hydrolysis of degraded egg plum gum affords D-galactose and an aldobionic acid, 6-D-glucuronosyl-D-galactose (I), the structure of which is identical with that of the aldobionic acid from gum arabic.

The periodate method of oxidation, which is known to cleave the carbon chain when adjacent hydroxyl groups are present, has been utilized under controlled conditions in this study of egg plum gum to gain some idea of the mode of union of the sugar units and the branching of the chains.[39] The results indicated that both D-galactose and L-arabinose residues are involved in union with other sugar units in such a fashion as to make them resistant to attack by periodate, thus showing that these particular units do not possess adjacent hydroxyl groups. This would appear to be a very useful application of an elegant method of oxidation already exploited with success in the carbohydrate field by C. S. Hudson and his coworkers.

6. *Mesquite Gum*

Reference should also be made in this section to the recent advances in the chemistry of mesquite gum, an exudate from mesquite trees[40,41] (*Prosopis juliflora* DC. and related species). The naturally occurring plant gum is the salt of a complex acid polysaccharide as is the case with

(38) J. K. N. Jones, *J. Chem. Soc.*, 1055 (1947).

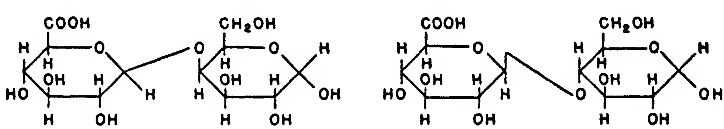
(39) V. C. Barry, T. Dillon and W. McGettrick, *J. Chem. Soc.*, 183, 578 (1942); V. C. Barry, *Nature*, **152**, 537 (1943).

(40) W. Procter, Jr., *Am. J. Pharm.*, **27**, 14, 223 (1855).

(41) C. Morfit, *Am. J. Sci.*, **19**, 263 (1855).

other plant gums.[42] It was shown to contain L-arabinose and D-galactose which could be isolated in the crystalline condition after hydrolysis of the gum with dilute mineral acid.[11] It was also recorded that the acid character of the gum was due to the presence of a uronic acid. The experiments also demonstrated that the uronic acid residues in mesquite gum differed from those in other gums for the reason that the former contained a methoxyl residue.[43]

The L-arabinose units are readily eliminated by hydrolysis with a weak acid and there remains, as with the other gums referred to above, a stable nucleus of degraded mesquite gum. During this mild hydrolysis only L-arabinose units are cleaved. Prolonged hydrolysis of the degraded gum with acid affords a mixture of aldobionic acids in which the D-glucuronic acid residues are characterized by the presence of a methoxyl group.[43a] The aldobionic acids have been shown to consist of a mixture of the monomethyl ether of 6-D-glucuronosyl-D-galactose (I) and 4-D-glucuronosyl-D-galactose (formula XXXVIII).[17,44] In view of the fact that degraded products of gum arabic have been shown to give a precipitin reaction with antipneumococcus sera, probably as a result of the presence of 6-β-D-glucuronosyl-D-galactose, it would be of interest to know whether degraded mesquite gum products containing a 1,4 linked aldobionic acid would be more active than products from degraded arabic acid, since 4-D-glucuronosyl-D-glucose (XXXIX) (cellobiuronic acid) appears to be responsible for the precipitin reaction of pneumococcus polysaccharides.[45]



XXXVIII XXXIX

An examination of the methanolysis products of methylated mesquite gum has revealed that they consist of five substances, of which the following four have been identified: 2,3,5-trimethyl-L-arabinose (XXV), 3,5-dimethyl-L-arabinose (XL), 2,4-dimethyl-D-galactose (XXI) and 2,3,4-trimethyl-D-glucuronic acid (VIII).

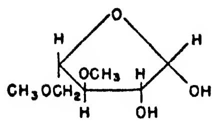
(42) E. Anderson, Lila Sands and N. Sturgis, *Am. J. Pharm.*, **97**, 589 (1925).

(43) E. Anderson and Louise Otis, *J. Am. Chem. Soc.*, **52**, 4461 (1930).

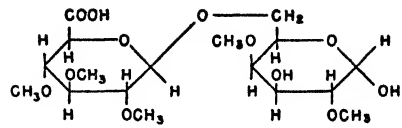
(43a) The methyl group has recently been located at C4; E. V. White, *J. Am. Chem. Soc.*, **70**, 367 (1948).

(44) E. V. White, *J. Am. Chem. Soc.*, **69**, 2264 (1947).

(45) W. F. Goebel, *J. Exp. Med.*, **68**, 469 (1938).



XL



XLI

These results show that all the L-arabinose units of mesquite gum possess the furanose form and the characterization of 3,5-dimethyl-L-arabinose demonstrates that the arabofuranose members are mutually linked by 1,2 glycosidic bonds. It will be recalled that in cherry gum and gum arabic, the arabofuranose units are linked by 1,3 glycosidic bonds while in damson gum the union is through a 1,5 link. There is some difference of opinion with regard to the relative proportions of the cleavage fragments but this is perhaps not surprising in view of the stability of the nucleus of methylated mesquite gum and the difficulty which attends the separation of the mixtures of cleavage fragments.[46,47] There is, however, sufficient agreement to be able to say that mesquite gum consists of a simple branched-chain acid-resistant nucleus composed of D-galactose and monomethyl-D-glucuronic acid units, all of which have pyranose structures, and to this nucleus are attached side chains consisting of L-arabinose units of the furanose type. The characterization of 2,4-dimethyl-D-galactose (XXI) as one of the cleavage fragments indicates the branched-chain nature of the polysaccharide, and the close relationship between the degraded acids of arabic, cherry and damson gums, in all of which branching occurs at a galactose residue. The similarity of this gum to those discussed above is also borne out by the fact that the uronic acid units were identified as 2,3,4-trimethyl-D-glucuronic acid as they were in the case of the methylated derivatives of gum arabic, cherry gum and damson gum. Furthermore, it is apparent that D-glucuronic acid and L-arabofuranose residues constitute terminal groups. In the case of mesquite gum no D-glucuronic acid residues were detected which were involved in union with other residues through position 4; in this particular the gum differs from the gums exuded by the cherry, damson and acacia trees.

Further experimental evidence is now available which enables the statement to be made that in mesquite gum the side chains of L-arabinose are attached to C3 of some D-galactose residues and to C6 of others.[48] The evidence for this observation is that the degraded mesquite gum,

(46) E. V. White, *J. Am. Chem. Soc.*, **68**, 275 (1946).
(47) J. I. Cunneen and F. Smith, *J. Chem. Soc.*, 1146 (1948).
(48) E. V. White, *J. Am. Chem. Soc.*, **69**, 622 (1947).

from which the arabinose units have been removed by mild hydrolysis, gives after methylation followed by hydrolysis 2,3,4-trimethyl- (XX) and 2,4,6-trimethyl-D-galactose (XIX) as well as 2,4-dimethyl-D-galactose (XXI). Methylated mesquite gum in which the L-arabinose units are still present gives rise to 2,4-dimethyl-D-galactose; no trimethyl derivative of D-galactose was detected. The 2,3,4-trimethyl- and 2,4,6-trimethyl-D-galactose have been recognized by their transformation into crystalline anilides and the 2,3,4-trimethyl-D-galactose has been further characterized by its conversion into crystalline 6-trityl-2,3,4-trimethyl-D-galactose anilide.[49] It is also clear from the identification of the two trimethyl-D-galactose derivatives that the main chains of D-galactose members of mesquite gum are mutually joined by 1,3 as well as 1,6 linkages.

From the observation that methyl 6-(2,3,4-trimethyl-β-D-glucuronosyl)-2,4-dimethyl-D-galactoside (compare XLI) is formed as a result of the action of methyl alcoholic hydrogen chloride upon methylated mesquite gum,[47] it might be said that the branches of degraded mesquitic acid consist of single units of D-glucuronic acid. The 2,4-dimethyl-D-galactose moiety of XLI would then constitute one of the D-galactose units in the main chain, from which it would follow that this galactose chain contains 1,3 linkages. On the other hand, if aldobionic acid residues constitute the side chains, as appears to be the case with arabic acid,[15] the 2,4-dimethyl-D-galactose unit of XLI will arise from a galactose unit in the aldobionic acid side chain and not from one in the main galactose chain. Should this be the case then the free hydroxyl group at C3 in the 2,4-dimethyl-D-galactose fragment of XLI represents the point of attachment of one side chain of L-arabofuranose units.[48]

7. *Gum Tragacanth*

Some progress has also been reported recently in the study of gum tragacanth, an exudate of shrubs belonging to the genus *Astragulus* (of the order *Leguminosae*) which grows in Southwest Europe and in the Middle East. The commercial importance of this gum, the jellying property of which is superior to that of other gums, has resulted in the artificial stimulation of its exudation by incisions made in the trunks of the trees.

Unlike the gums referred to above, which are essentially homogeneous, this gum is a complex mixture, a fact recognized by O'Sullivan who had subjected it to very careful fractionation. Hydrolysis of the crude gum with dilute mineral acid has been shown to afford L-arabinose, D-xylose

(49) E. V. White, *J. Am. Chem. Soc.*, **64**, 1510 (1942).

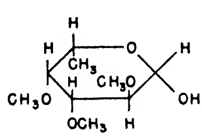
and L-fucose.[50] Hilger and Dreyfus[51] isolated D-galactose as well as L-arabinose from gum tragacanth while O'Sullivan in his paper[5] made the further suggestion that acid degradation products from the gum bore some relationship to the degraded products from gum arabic[26] and gedda gum,[3] which he had previously studied.

The crude gum tragacanth is a mixture of the salt of a complex acid polysaccharide and a neutral polysaccharide composed principally of L-arabinose residues.[52] Starch is also present in the gum.[5] The acid character of this gum is due to units of D-galacturonic acid and not D-glucuronic acid and it is of interest to note that in its ability to form gels it resembles pectin and the plant mucilages, which also contain D-galacturonic acid.

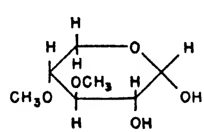
The neutral polysaccharide is of the nature of an araban, but whether this polysaccharide is in physical admixture with the acid complex or is chemically bound to it is not yet certain.

Methylation of crude gum tragacanth by the methyl sulfate method affords the complex acid polysaccharide as a methyl derivative. It is this portion of the gum, referred to as tragacanthic acid, which resembles plant gums. Treatment of the methylated tragacanthic acid with methyl alcoholic hydrogen chloride affords a mixture of the methyl glycosides of the following substances: 2,3,4-trimethyl-L-fucose (XLII), 2,3,4-trimethyl-D-xylose (XXXII), 3,4-dimethyl-D-xylose (XLIII), 2,3-dimethyl D-galacturonic acid (XLIV) and a monomethyl-D-galacturonic acid.

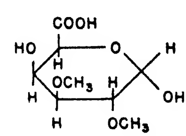
The mode of union of these units in the complex methylated tragacanthic acid is obvious from their identification but the order of combination is still obscure. The 2,3,4-trimethyl-D-xylose (XXXII) and 2,3,4-trimethyl-L-fucose (XLII) must arise from terminal residues in the complex, while the 3,4-dimethyl-D-xylose (XLIII) must be involved in union



XLII



XLIII



XLIV

at positions 1 and 2 with other residues (see later ref. 99). The points of attachment of the various cleavage fragments are shown in the formulas by the free hydroxyl groups. It is also clear that the 2,3-dimethyl-D-

(50) J. A. Widtsoe and B. Tollens, *Ber.*, **33**, 132 (1900).
(51) A. Hilger and W. E. Dreyfus, *Ber.*, **33**, 1178 (1900).
(52) Sybil P. James and F. Smith, *J. Chem. Soc.*, 749 (1945).

galacturonic acid (XLIV) is derived from galacturonic acid units of methylated tragacanthic acid which are joined to other residues through positions 1 and 4, as they are in pectic acid.[53,54] The formation of the crystalline methyl ester of the methyl monomethyl-β-D-galactopyruronoside clearly arises from a galacturonic acid unit which is involved in the branching of the chains in the molecule and it is probable that these are the galacturonic acid units to which the side chains of L-fucose and D-xylose are attached. The constitutional significance of the isolation of this monomethyl compound is thus analogous to that already deduced from the formation of 2,4-dimethyl-D-galactose (XXI) from the methylated derivatives of damson gum, cherry gum, mesquite gum and gum arabic. The 2,3-dimethyl-D-galacturonic acid was actually identified as a crystalline derivative of a galactofururonoside whilst the monomethyl-D-galacturonic acid was obtained as a pyruronoside. The difference in ring structure of these two uronic acid derivatives is not to be taken as an indication that pyranose and furanose forms of D-galacturonic acid pre-exist in the complex gum. In point of fact, the relatively high stability of tragacanthic acid and its methyl derivative and the high positive rotation have led to the suggestion that the uronic acid residues present in the gum are all of the pyranose variety. Although the monomethyl-D-galacturonic acid was not completely identified, it must be the 2- or 3- or 4-monomethyl derivative since methylation gives the crystalline methyl ester of methyl 2,3,4-trimethyl-β-D-galacturonoside. Its isolation, taken together with the identification of the terminal residues as 2,3,4-trimethyl-D-xylose and 2,3,4-trimethyl-L-fucose,[55,56] shows clearly that this gum acid is similar to the other plant gums so far studied in detail, in possessing a branched chain structure.

Sufficient experimental evidence has thus been accumulated to enable the main structural features of plant gums to be deduced, but it is very evident that much more work remains to be done before a unique structure can be assigned to any one plant gum. The problems involved appear at the outset to be somewhat forbidding but it seems to us not unlikely that while plant gums differ in detail they may be assembled on one or perhaps two common structural frameworks which are relatively simple. The elucidation of the structure of such a common framework would be of great significance inasmuch as it may well be that a knowledge of the size and shape of such a framework would contribute to a better understanding of life processes in general.

(53) G. H. Beaven, E. L. Hirst and J. K. N. Jones, *J. Chem. Soc.*, 1865 (1939).
(54) Sybil P. Lucett and F. Smith, *J. Chem. Soc.*, 1506 (1940).
(55) Sybil P. James and F. Smith, *J. Chem. Soc.*, 746 (1945).
(56) O. T. Schmidt, W. Mayer and A. Distelmaier, *Ann.*, **555**, 26 (1943).

Although the isolation and identification of new disaccharides, trisaccharides and tetrasaccharides and their derivatives, either by acid hydrolysis or by controlled oxidative degradation,[39a,39b] would be of great help in these studies it would appear to be worth while to develop other indirect methods of approach involving the use of enzymes capable of effecting scission at specific points in the molecular complex. Better methods for the quantitative separation of sugars and their derivatives are in the process of development[57–62] and it is not unlikely that in the near future it will be possible to derive formulas not only for plant gums but for the many related complex polysaccharides.

II. Mucilages

1. *Occurrence, Function and Isolation*

The plant mucilages are polysaccharides which form colloidal solutions in water from which they can often be precipitated with ammonium sulfate, sodium chloride and the usual protein precipitants. They differ from pectin in that they do not form jellies, but some seaweed mucilages are exceptions to this rule, an example being agar. They usually contain D-galactose as part of the molecule. The mucilages can exist either as a secondary membrane thickening material or as an intracellular substance, and they may therefore be differentiated into membrane mucilages or cell content mucilages. Membrane mucilages occur in the root (*Althaea*), the cortex (*Cinnamonum*), the stalk (*Tragacanth*), the leaves (*Buccu*), the flowers (*Malvaceae, Tilia*), the endosperm (*Trigonella*) and in the seed shell (cocoa). In seaweeds the mucilage is intercellular (*Laminaria, Carragheen*); in the succulents (*Aloe, Euphorbiaceae*) the mucilage occurs in the cell content. In several bulbs (*Scilla*) and in *Orchis* (salep) mucilage cells occur.

The mucilage is sometimes a food reserve (*Linum, Cruciferae*) and in plants living in dry climates it may act as a water reservoir. The formation of resins and oils is said to be the function of a mucilaginous membrane.

(57) W. S. Reich, *Compt. rend.*, **208**, 589, 748 (1939); *Biochem. J.*, **33**, 1000 (1939).

(58) G. H. Coleman, A. G. Farnham and A. Miller, *J. Am. Chem. Soc.*, **64**, 1501 (1942); G. H. Coleman and C. M. McCloskey, *ibid.*, **65**, 1588 (1943); G. H. Coleman, D. E. Rees, R. L. Sundberg and C. M. McCloskey, *ibid.*, **67**, 381 (1945).

(59) J. K. N. Jones, *J. Chem. Soc.*, 333 (1944).

(60) D. J. Bell, *J. Chem. Soc.*, 473 (1944).

(61) B. W. Lew, M. L. Wolfrom and R. M. Goepp, Jr., *J. Am. Chem. Soc.*, **68**, 1449 (1946); W. W. Binkley and M. L. Wolfrom, *ibid.*, **68**, 1720 (1946); L. W. Georges, R. S. Bower and M. L. Wolfrom, *ibid.*, **68**, 2169 (1946).

(62) A. E. Flood, E. L. Hirst and J. K. N. Jones, *Nature*, **160**, 86 (1947).

Some mucilages are stained blue, some have the reactions of cellulose (*Cydonia*) and some are only stained yellow with cellulose-staining reagents.

If the mucilage occurs on the outside of the seed coating then, for isolation, extraction with water suffices; if in the endosperm (as for *Foenum graecum*) or in the tuber tissue (salep) then the seed or tubers must be powdered.

The hydrolysis products of mucilages from orchids and from cell wall thickening material of numerous endosperms (Palm, *Strychnos*, *Leguminosae*) usually contain D-mannose and D-galactose. These mucilages are reserve polysaccharides and are split by the enzyme seminase, as well as by the enzymes occurring in *Aspergillus niger*, *A. fuscus* and in barley malt. Mannan occurs in the root of *Conophalus Konjaku* and the mucilage in *Hydrangea paniculata* contains D-galactose, D-mannose and L-arabinose. This mucilage is hydrolyzed by the enzymes in *Bacillus mesentericus vulgatus*. Seaweed mucilages of the type of agar and gelose are hydrolyzed by the enzymes in *Bacillus gelaticus*, an organism found in seawater.

2. *Classification*

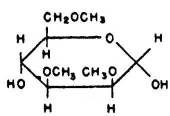
The mucilages may be divided roughly into three groups, according to their chemical characteristics. (a) The neutral polysaccharides, consisting of one or more sugar residues joined together through their reducing groups with the formation of substances of high molecular weight. (b) The polysaccharides containing uronic acid residues as well as other sugar residues. (c) The mucilages which occur in seaweeds and which consist in the main of the salts of sulfate esters of sugar derivatives of high molecular weight.

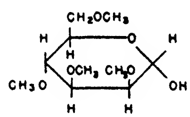
Very little is known of the detailed chemistry of any member of these three groups. In many cases mucilages have been isolated and submitted to a cursory examination with the object of determining the sugars present in them. In some cases, attempts have been made to determine the approximate amount of sugars present. Very few mucilages, however, have been tested for homogeneity or have been submitted to detailed examination with the object of determining the mode and order of union of the component sugars.

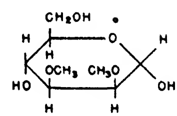
3. *Neutral Mucilages*

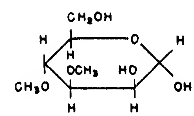
One of the first mucilages to be examined in any detail was that from the *Orchidaceae*, namely, salep mannan or mucilage. This material may be extracted from the powdered tuber with cold water from which it is precipitated with alcohol. It was early shown that D-mannose is the

sole constituent.[63] Methylation followed by hydrolysis yielded 2,3,6-trimethyl-D-mannose (XLV) and a very small amount of tetramethyl-D-mannopyranose[64] (XLVI), indicating that the mucilage is a linear polymer of 1,4-linked D-mannose residues, the sugar probably being present in the pyranose form and linked glycosidically through β-links since the mannan (XLVII) had a low negative rotation ($[\alpha]_D$ *ca.* $-30°$). This mucilage closely resembles ivory nut mannan[65] in structure but some of its physical properties (for example, solubility in water) are very different. The mucilaginous material from the foenugreek (*Trigonella foenum-graecum*) has been identified as a mannogalactan and may exist as a complex silico-phosphoric ester in the seed.[66] This mucilage contains the same sugars, D-galactose and D-mannose, as are present in the mucilages in Guar, locust bean, honey locust, flame tree seed, Kentucky coffee bean, Paloverde, tara, huizache, *Sophora Japonica*,[67] "gum" gatto[68] (carob seed gum, locust bean gum, gum tragon, St. John's bread) and lucerne seed.[69]


XLV


XLVI


XLVIII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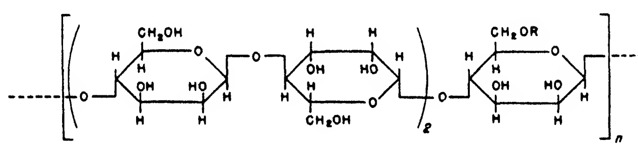

R = H; XLVII

R = D-galactopyranose; XLIX

These last two mucilages have been examined in some detail. Gum gatto in some respects has a structure which resembles that of salep mannan and ivory nut mannan. Lucerne seed mucilage, on the other

(63) A. Hilger, *Ber.*, **36**, 3197 (1903); H. Pringsheim and G. Liss, *Ann.*, **460**, 32 (1928); *cf.* Y. Kinoshito, *Bull. Coll. Agric. Imp. Univ. Tokyo*, **2**, 205 (1895).

(64) F. Klages and R. Niemann, *Ann.*, **523**, 224 (1936).

(65) J. L. Baker and T. H. Pope, *J. Chem. Soc.*, **77**, 696 (1900); K. Hess and M. Lüdtke, *Ann.*, **456**, 201 (1927); K. Klages, *ibid.*, **509**, 159 (1934); **512**, 185 (1936).

(66) K. M. Daoud, *Biochem. J.*, **26**, 255 (1932); C. R. H. Iyer and B. N. Sastri, *J. Indian Inst. Sci.*, **16A**, 88 (1933).

(67) L. E. Wise and J. W. Appling, *Ind. Eng. Chem., Anal. Ed.*, **16**, 28 (1944).

(68) A. Spada, *Atti Soc. Nat. Mat. Modena*, **70**, 20 (1939).

(69) E. L. Hirst, J. K. N. Jones and (Mrs.) W. O. Walder, *J. Chem. Soc.*, 1443 (1947).

hand, has a very different type of structure. Gum gatto[70] on acidic hydrolysis yields D-galactose (*ca.* 16%) and D-mannose (84%) only; hydrolysis with enzymes such as those present in takadiastase or "pectinol 10M" leads to the formation of D-mannose in low yield. The polysaccharide is readily methylated with sodium hydroxide and methyl sulfate and the optical rotation of the resultant methyl ether ($[\alpha]_D$ *ca.* $-11°$ in water) indicates that the linkage between the mannose residues is mainly of the β-type. On hydrolysis of methylated gum gatto three main products were identified: (a) 2,3,4,6-tetramethyl-D-galactose (XI), (b) 2,3,6-trimethyl-D-mannose (XLV) and (c) 2,3-dimethyl-D-mannose (XLVIII) in the approximate molecular ratio 1:4:1. All the D-galactose was therefore present in the polysaccharide as an end group and was attached to a D-mannose residue either through C6 or C4. One of the structures which will accommodate these facts is given in XLIX. Confirmation of this suggested structure was obtained from a study of the action of potassium periodate on the gum. This reagent, diagnostic for α-glycols, reacts with the gum with the formation of one mole of formic acid per six sugar residues, and with the consumption of 1.16 moles of periodate per mole of sugar, thus proving the presence of one α-β-γ triol, and five α-glycol residues in each of six sugar residues. Hydrolysis of the oxidised polysaccharide yielded traces only of D-mannose on the paper chromatogram,[71] showing that the reagent had destroyed practically all the sugar residues and that D-galactose or D-mannose linked through C3 or through C2 and C4 could be present in traces only. Gum gatto appears to be similarly constituted to locust bean mucilage. This substance has been investigated in detail by Smith[72] who has isolated 2,3,4,6-tetramethyl-D-galactose (XI), 2,3,6-trimethyl-D-mannose (XLV) and 2,3-dimethyl-D-mannose (XLVIII) from the hydrolysis products of the methylated mucilage.

The mucilage in lucerne seed (extracted from the powdered seed) has been investigated by May and Schulze[73] who showed that it contained D-galactose and D-mannose and that it gave an insoluble copper complex. These authors were of the opinion that the ratio of sugars was one to one but later workers[69] using a different sample of the mucilage arrived at a ratio of two parts of D-galactose to one part of D-mannose. Little is known of the fine structure of this mucilage. Methylation experiments, however, showed that D-galactose was an end group and that D-galactose residues linked through C1 and C3 and D-mannose residues linked

(70) E. L. Hirst and J. K. N. Jones, *J. Chem. Soc.*, 1278 (1948).
(71) S. M. Partridge, *Nature*, **158**, 270 (1946).
(72) F. Smith, *J. Am. Chem. Soc.*, **70**, 3249 (1948).
(73) F. May and K. Schulze, *Z. Biol.*, **97**, 201 (1936).

through C1, C2 and C6 also occurred since 2,3,4,6-tetramethyl-D-galactose (XI), 2,4,6-trimethyl-D-galactose (XIX) and 3,4-dimethyl-D-mannose (L) were isolated from the products of hydrolysis of the methylated mucilage. This mucilage is of rather more complicated structure than the galactomannans of gum gatto and locust bean gum and does not seem to be related to them since 1,4-linked D-mannose residues do not appear to be present in it.

Many other mucilaginous polysaccharides of this type have been examined (see Table IV) but little is known of their detailed structure.

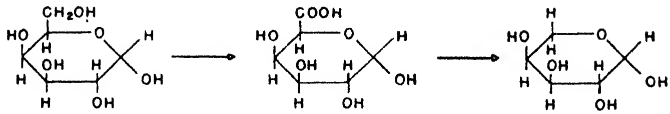
TABLE IV
Constituent Sugars of Various Neutral Mucilages

Source	*Sugars present*	*Reference*
Sterculia plantanifolia (Young shoots)	L-arabinose, D-galactose	74
Colocasia antiquorum (Tuberous roots)	D-glucose	74
Vitis pentaphylla (Stems and leaves)	D-galactose	74
Opuntia (Fleshy stems)	D-galactose	74
Oenothera jaquinii (Stems and leaves)	D-galactose and L-arabinose	74
Kadzura japonica (Stems and leaves)	D-galactose and L-arabinose	74
Opuntia vulgaris	D-galactose and L-arabinose	75
Abelmoschus manihot	D-galactose, L-arabinose and L-rhamnose	76
Capsicum seeds	D-galactose and L-arabinose	77
Hydrangea paniculata Sieb. (bark)	D-galactose, L-arabinose and methyl pentose	78
Anagyris foetida (seeds)	D-galactose and L-arabinose	79
Polygonatum officinale Alb. (rhizome)	D-glucose, L-arabinose and D-fructose	80
Actinidia callosa Lindl. var. ruf. Makino (bark)	D-galactose and L-arabinose	81
Viola flowers		82

It will be observed that a large proportion of these mucilages contain D-galactose and L-arabinose, two of the easiest sugars to detect. Whether these two sugars comprise the main bulk of the neutral mucilages or whether other more difficultly detectable sugars are present in small amounts remains to be determined. It may be significant, however, that L-arabinose, which may be derived from D-galactose by way of D-galac-

(74) K. Yoshimura, *Bull. Coll. Agric. Imp. Univ. Tokyo*, **2**, 207 (1895).
(75) V. Harlay, *J. Pharm. Chim.*, [VI] **16**, 193 (1902).
(76) T. Ozawa, *J. Chem. Ind.* (Japan), **25**, 389 (1922).
(77) B. V. Bitto, *Landw. Versuchs. Stat.*, **46**, 309 (1895).
(78) S. Komatsu and H. Ueda, *Mem. Coll. Sci. Kyoto Imp. Univ.*, **8**, 51 (1925).
(79) P. Condorelli, *Ann. chim. applicata*, **15**, 426 (1925); **18**, 313 (1928).
(80) B. Gaal, *Ber. Ungar. Pharm. Ges.*, **3**, 133 (1927).
(81) Y. Kihara, *J. Agr. Chem. Soc.* (Japan), **14**, 733 (1938).
(82) P. Balavoine, *Pharm. Acta Helv.*, **21**, 19 (1946).

turonic acid by a process of oxidation and decarboxylation, is so often associated with D-galactose (see LI).



LI

4. *Mucilages Containing Uronic Acid Residues*

In this class of materials is grouped the majority of the seed mucilages, the acidity of which is due to a uronic acid (usually D-galacturonic acid) or to a methyl ether derivative of a uronic acid. This presence of D-galacturonic acid as the acidic component of the polysaccharide differentiates the mucilages from the gums, the acidity of the majority of which is due to the presence of D-glucuronic acid, or to one of its methyl ether derivatives (see above).

The origin and function of these mucilages is a matter of some dispute; some authors consider that the mucilages arise in the plant in specialized cells which are capable of converting cell wall material through hydrocellulose into mucilage[83] while others[84] believe that the contents of many cells in the growing regions are converted into a mucilage, and that the cell wall is not involved. It is generally admitted, however, that the mucilage may act as a food reserve[85,86] and as a means of storing water.[84,85,87] The origin of the mucilage in the seeds of *Linum usitatissimum* L. has been examined in great detail by Jaretzky and Ulbrick[88] who came to the conclusion that starch is converted into an intermediate compound without the loss of granular structure, and that this material is later converted into mucilage. Many members of this group of mucilages have been analysed (see Table V) and the constitution of some of them is known in fair detail. It will be observed that these polysaccharides (Table V) contain, on the whole, a wider variety of sugars than are present in the neutral mucilages (Table IV) and that D-galactose is again a very commonly occurring component. Quince seed[89] mucilage

(83) F. E. Lloyd, *Am. J. Bot.*, **6**, 156 (1919).

(84) E. G. Stewart, *Bull. Torrey Botan. Club*, **46**, No. 5, 157 (1919); *Expt. Sta. Record*, **43**, 226; *Chem. Abstracts*, **15**, 2898 (1921).

(85) L. Montemartini, *Lavori ist. botan. Palermo*, **5**, 45 (1924).

(86) C. Ravenna and M. Zamorani, *Atti R. Acad. Lincei*, (V), **19**, ii, 247 (1910).

(87) J. B. McNair, *Am. J. Botany*, **19**, 168 (1932); J. Rae, *Pharm. J.*, **151**, 241 (1943).

(88) R. Jaretzky and H. Ulbrick, *Arch. Pharm.*, **272**, 796 (1934); R. Jaretzky and E. Bereek, *ibid.*, **276**, 17 (1938).

(89) W. Kirchener and B. Tollens, *Ann.*, **175**, 205 (1874); Alice G. Renfrew and L. H. Cretcher, *J. Biol. Chem.*, **97**, 503 (1932).

TABLE V

Constituents of Various Mucilages that Contain Uronic Acid Residues

Mucilage	*Sugars present*	*Acid present*	*Reference*
Linseed	Xylose, arabinose, galactose, glucose	Not detected	63, 90
Linseed	D-xylose, L-galactose, L-rhamnose	D-galacturonic	9, 91
White mustard seed	D-xylose, L-arabinose, methyl pentose (combined cellulose)	D-glucuronic and D-galacturonic	92
Cress seed	L-arabinose, D-galactose, L-rhamnose, D-xylose (combined cellulose)	D-galacturonic	93
Kadzura Japonica, Dun. (stem)	D-xylose	D-glucuronic	94
Scaphium affine, Pierre (fruit)	L-arabinose, acetic acid	D-galacturonic	95
Brasenia schreberi, Gmel. (leaves)	D-galactose, D-mannose and L-arabinose	D-glucuronic	96
Plantago psyllium (seed)	L-arabinose, D-xylose	D-galacturonic	97
Plantago fastigiata (seed)	L-arabinose, D-xylose	D-galacturonic	98
Plantago lanceolata (seed)	D-galactose, D-xylose	D-galacturonic	99
Plantago arenaria	D-xylose, L-arabinose, D-galactose	D-galacturonic	100
Ulmus fulva (bark)	L-rhamnose, D-galactose	D-galacturonic	8, 101
Pachyra affinia (roots)	?	?	102

(90) K. Neville, *J. Agric. Sci.*, **5**, 113 (1913).

(91) E. Anderson and J. A. Crowder, *J. Am. Chem. Soc.*, **52**, 3711 (1930); E. Anderson, *J. Biol. Chem.*, **100**, 249 (1933); E. Anderson and H. J. Lowe, *ibid.*, **168**, 289 (1947).

(92) K. Bailey and F. W. Norris, *Biochem. J.*, **26**, 1609 (1932).

(93) K. Bailey, *Biochem. J.*, **29**, 2477 (1935).

(94) K. Nishida, H. Hashima and T. Fukamizu, *J. Agr. Chem. Soc. Japan*, **10**, 1001 (1934); **11**, 261 (1935).

(95) H. Nakahara, *J. Agr. Chem. Soc. Japan*, **11**, 310 (1935).

(96) H. Nakahara, *J. Agr. Chem. Soc. Japan*, **16**, 140, 876 (1940).

(97) E. Anderson and M. Fireman, *J. Biol. Chem.*, **109**, 437 (1935); H. W. Youngken, *Am. J. Pharm.*, **106**, 157 (1934).

(98) E. Anderson, L. A. Gillette and M. G. Seeley, *J. Biol. Chem.*, **140**, 569 (1941).

(99) J. Mullan and E. G. V. Percival, *J. Chem. Soc.*, 1501 (1940).

(100) W. A. G. Nelson and E. G. V. Percival, *J. Chem. Soc.*, 58 (1942).

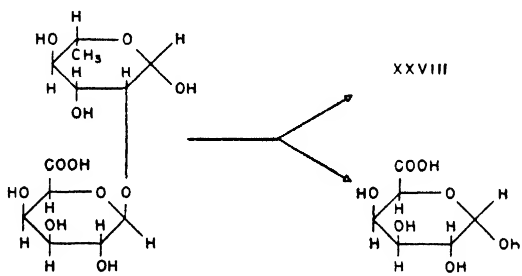
(101) (a) E. Anderson, *J. Biol. Chem.*, **104**, 163 (1934); (b) R. E. Gill, E. L. Hirst and J. K. N. Jones, *J. Chem. Soc.*, 1025 (1946).

(102) T. M. Meyer and J. W. van Dalfsen, *Arch. Rubbercultuur*, **23**, 172 (1939).

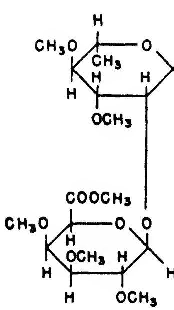
is unusual in that it is combined with cellulose, this combination conferring solubility on the usually insoluble cellulose. Hydrolysis of the mucilage causes the cellulose to separate in a fibrous form. This property of conferring solubility on the otherwise insoluble cellulose molecule is also possessed by the mucilages of white mustard seed[92] and cress seed.[93] It may be that this property is one of the reasons why mucilages are so useful in improving the quality of paper. The methods used in isolating and investigating these three polysaccharides are general methods which are widely employed in the examination of the polysaccharides containing uronic acid residues[103] and they will therefore be described in some detail giving, as an example, the isolation of white mustard seed mucilage. The crude mucilage, isolated by extraction of the seeds with cold water, is filtered through cloth and the gel precipitated by the addition of alcohol to the cold aqueous solution. The resultant crude mucilage is then dissolved in water and fractionally precipitated by the addition of barium hydroxide solution. In some cases the mucilage is precipitated as a copper complex. The various fractions may then be analyzed for uronic acid, pentosan and methyl pentosan content, or, after hydrolysis, the sugars may be identified and separated quantitatively on the paper chromatogram.[62] The linkages joining the pentose and methyl pentose residues in the mucilages are usually hydrolyzed much more easily than those connecting the hexose and uronic acid residues; consequently, hydrolysis of a mucilage of this type (b) may lead to the isolation of fragments built up of two or more sugar residues, one of which will be a uronic acid. For example, hydrolysis of the mucilage from white mustard seed leads to the isolation of L-arabinose, D-galactose and a disaccharide built up of L-rhamnose and D-galacturonic acid. This disaccharide is an aldobionic acid, a general name given to a disaccharide composed of a sugar and a uronic acid residue. Every aldobionic acid so far isolated from the hydrolysis of natural materials has been found to be built up in such a way that the reducing power of the disaccharide is due to the sugar portion of the molecule, the aldehyde group of the uronic acid being protected by glycoside formation. Very rarely is a disaccharide that is built up of two neutral sugars encountered amongst the products of hydrolysis of a mucilage or gum (see, however, gum arabic, page 250). The identification of an aldobionic acid or of any degraded fragment isolated from the hydrolysis of a mucilage will give some information on the fine structure of the polysaccharide. Linseed mucilage on hydrolysis yields L-galactose, D-xylose and an aldobionic acid (LII), which on further hydrolysis gives D-galacturonic acid (LIII) and L-rhamnose (XXVIII) in equimolecular

(103) E. Anderson and Lila Sands, *Advances in Carbohydrate Chemistry*, **1**, 329 (1945).

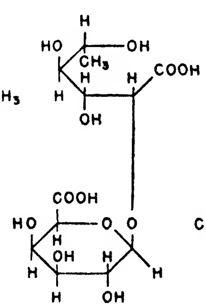
proportions. Oxidation of this aldobionic acid (LII) with hypoiodite yields a product (LIV) which still gives positive tests for the uronic acid residue. It follows therefore that the reducing aldehyde group in LII is part of the rhamnose molecule and that the aldehyde group of the galacturonic acid residue is protected by glycoside formation.[91] The position of the linkage between the galacturonic acid residue and the L-rhamnose remains undecided; it may be between the glycosidic hydroxyl of the uronic acid residue and the hydroxyl group on either C2, C3, C4 or C5 of the L-rhamnose residue. Moreover, the galacturonic acid may be in the pyranose (six ring) or furanose (five ring) form. However, the difficulty encountered in hydrolyzing the glycosidic linkage in the aldobionic acid indicates that the pyranose ring is more probable. Further



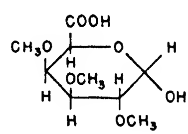
LII LIII



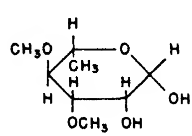
LV



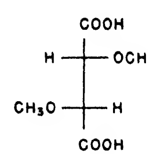
LIV



LVI



LVII



LVIII

insight into the structure of the aldobionic acid can be gained from methylation experiments. If all the free hydroxyl groups in the disaccharide are converted into the corresponding methyl ethers, then hydrolysis of the product (LV) will indicate the point of junction between the two sugar residues. The products isolated were 2,3,4-trimethyl-D-galacturonic acid (LVI) and 3,4-dimethyl-L-rhamnose (LVII). The

galacturonic acid derivative (LVI) had earlier been characterized[104] and was known to be of the pyranose type. The dimethyl rhamnose derivative (LVII) had also been prepared earlier[105] and its structure proved by oxidation to dimethyl-L-tartaric acid (L(+)(*threo*)-dimethoxysuccinic acid) (LVIII). The only points of attachment between the uronic acid residue and the methyl pentose sugar are therefore C2 or C5 if a reducing aldobionic acid is to be formed. It is exceedingly unlikely that the linkage is between C1 and C5 since the L-rhamnose residue would then possess a three membered ring (between C1 and C2). It is inferred, therefore, that the linkage between the two sugars is between C1 of the uronic acid residue and C2 of the methyl pentose residue. In a similar manner the structure of the aldobionic acid isolated from the products of hydrolysis of *Ulmus fulva* has been shown to be LII. Aldobionic acids containing D-galacturonic acid and L-rhamnose also occur in the products of hydrolysis of the mucilages from white mustard seed, cress seed and *Plantago lanceolata.*

Further information on the structure of the mucilage may be obtained from an examination of the products produced on hydrolysis of the methylated materials. The conversion of these polysaccharides into their corresponding methyl ether derivatives without considerable degradation is not an easy matter, especially when the uronic acid content of the mucilage is high. In some cases it has been found advantageous to use the Menzies methylation procedure[34] rather than the standard Haworth reaction.[8,106] Hydrolysis of the methylated mucilage yields a variety of products (see Table VI). The separation and quantitative estimation of these methylated sugar derivatives is attended with many experimental difficulties, and, even if the separation and estimation has been satisfactorily completed, it is impossible, without much further evidence, to say how and in what order these various sugar fragments are fitted together.

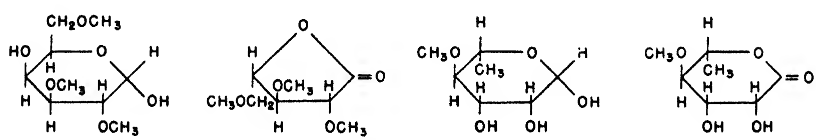
Only three mucilages of type (b) have been investigated by the methylation procedure. These are the polysaccharides from *Ulmus fulva,*[101] *Plantago lanceolata*[99] and *Plantago arenaria.*[100] Since the structure of an aldobionic acid from *Ulmus fulva* is known, rather more information concerning the fine structure of this mucilage may be gained from the identification and estimation of the various sugar derivatives produced on hydrolysis of the methylated product (see Table VI). 2,3,4,6-Tetramethyl-D-galactose (XI) and 2,4,6-trimethyl-D-galactose

(104) R. S. Tipson, C. C. Christman and P. A. Levene, *J. Biol. Chem.*, **120**, 597 (1937).
(105) W. N. Haworth, E. L. Hirst and E. J. Miller, *J. Chem. Soc.*, 2469 (1929).
(106) W. N. Haworth, E. L. Hirst and H. A. Thomas, *J. Chem. Soc.*, 825 (1931).

TABLE VI

Source	*Sugar*	*Derivative*
Slippery elm[101] (*Ulmus fulva*)	D-galacturonic acid	2,3-dimethyl- and 2,3,4-trimethyl-.
	D-galactose	2,3,4,6-tetramethyl-, 2,4,6- and 2,3,6-trimethyl-.
	L-rhamnose	3,4-dimethyl- and 4-methyl-.
Plantago lanceolata[99]	D-galacturonic acid	?
	D-galactose	2,4,6-trimethyl-.
	D-xylose	2,3,4-trimethyl- and 3,4-dimethyl-.
	methyl pentose	?
Plantago arenaria[100]	D-galacturonic acid	?
	D-galactose	2,3,4-tetramethyl-.
	D-xylose	2,3,4-trimethyl-, 3,4-dimethyl- and 2-methyl-.
	L-arabinose (an aldobionic acid consisting of D-galacturonic acid and D-xylose)	?

(XIX) were characterized as their crystalline anilides, while 2,3,6-trimethyl-D-galactose (LIX) was characterized as the crystalline furanolactone (LX). 4-Methyl-L-rhamnose (LXI) was obtained crystalline and was converted into the crystalline pyranolactone (LXII). Esti-



LIX LX LXI LXII

mates only could be made of the amounts of the sugar derivatives produced on hydrolysis of the methylated mucilage. These estimates were obtained from an examination of the boiling point, refractive index, methoxyl content and optical rotation of the various fractions obtained on distillation, as well as from quantitative isolation of crystalline derivatives of known constitution.[101] Even with this information no unique structure can be postulated for the mucilage, as many problems remain unsolved. For example, 2,3,6-trimethyl-D-galactose may arise from a galactopyranose residue linked through C1 and C4 or from a galactofuranose residue linked through C1 and C5. It is unknown whether the mucilage possesses any degree of symmetry and whether it consists of a repeating unit, repetition of which leads to the formation of a polymer, or whether the sugar residues are distributed at random

throughout the molecule. These and other problems with less information available exist in determing the structure of the mucilage of *Plantago lanceolata* and *Plantago arenaria*.

5. *Seaweed Mucilages*

The third group of mucilages are all isolated from the seaweeds and are, in the main, salts of sulfate esters of polysaccharides, a large number of which have been isolated (see Table VII). In only one or two

TABLE VII

Source	*Components*	*Reference*
Chrondus elatus	D-galactose, L arabinose and "floridose"	107
Gloiopeltis furcatea var. *coliformis*	L-fucose, D-galactose and L-arabinose	107
Irideae laminarioides var. *cornucopiae*	D-galactose, L-arabinose and "floridose," sulfate	107
Chrondus crispus *Ceramium rubrum* *Delassaria sanguinea* *Delassaria elata* *Polysiphonia fastigiata* *Plumaria elegans*	D-galactose, sulfate and D-glucose	108
Ascophylum nodosum	L-fucose and D-mannose	109
Laminaria flexicaulis	D-glucose, D-galactose	110
Gelidium pacificum Okam.	D-galactose	111
Chrondus ocellatus Holmes *Fucus typicus* and *Fucus giganteus* Okam. *Iridaea laminarioides.*	D-galactose, methyl pentose, 2-keto-hexonic acid (?), sulfate, D- and L-erythrose	112
Laminaria and *Fucus* sp.	Fucoidin (Fucosan) L-fucose, sulfate	113
Macrocystis pyrifera	L-fucose, sulfate	114
Chrondus crispus (Carragheen)	D-galactose, sulfate	115
Carragheen	2-keto-D-gluconic acid	116
Dilsea edulis	D-galactose, sulfate	117, 119
Gigartina stellata	D-galactose, sulfate	118
Rhodymenia palmata	D-xylose	119
Laminaria cloustonii (Laminarin)	D-glucose	120*a*
Laminaria digitata	D-glucose	120*b*, 120*c*
Gelidium latifolium *Gelidium crinale* *Gracilara confervoides* (Agar-agar)	D-galactose, L-galactose, sulfate	121

(107) E. Takahashi, *J. Coll. Agr. Hokkaido Imp. Univ.*, **8,** 183 (1920); T. Tadokoro and T. Saito, *J. Soc. Chem. Ind. Japan,* **38,** suppl. binding, 270 (1935). See also

cases, however, is anything known of their detailed structure. Practically all of them contain D-galactose and in one or two the very rare 2-keto-D-gluconic acid (LXIII), a derivative of fructose, has been detected. Some appear to contain uronic acid residues but since a 2-keto-hexonic acid will also give the reactions of a uronic acid it is possible that this latter type of acid is more commonly occurring in the seaweed polysaccharides than is generally realized.

W. Z. Hassid, *J. Am. Chem. Soc.*, **55,** 4163 (1933); **57,** 2046 (1935).

(108) P. Haas, *Biochem. J.*, **15,** 469 (1921); P. Haas and B. Russell Wells, *Yearbook British Pharm. Conference*, 644 (1923); *Biochem. J.*, **23,** 425 (1929).

(109) R. H. F. Manske, *J. Biol. Chem.*, **86,** 571 (1930).

(110) Z. Gruzewska, *Compt. rend.*, **173,** 52 (1921).

(111) Y. Uyeda, *J. Soc. Chem. Ind. Japan*, **32,** 568 (1929), suppl. binding, **32,** 175B (1929).

(112) T. Mori and Y. Tsuchiya, *J. Agr. Chem. Soc. Japan*, **14,** 609, 616 (1938); T. Mori, *ibid.*, **15,** 1070 (1939); T. Mori and Y. Tutiya, *ibid.*, **15,** 1065 (1939). See also T. Yanagigawa and T. Yosida, *Repts. Imp. Ind. Research Inst., Osaka, Japan*, **17,** No. 5 (1936); 20, No. 5 (1939); T. Tadakoro and K. Yoshimura, *J. Chem. Soc. Japan*, **56,** 188, 655 (1935).

(113) H. Kylin, *Z. physiol. chem.*, **83,** 171 (1913).

(114) D. R. Hoagland and L. L. Lieb, *J. Biol. Chem.*, **23,** 287 (1915); W. L. Nelson and L. H. Cretcher, *ibid.*, **94,** 147 (1931).

(115) T. Dillon and A. McGuinness, *Sci. Proc. Roy. Dublin Soc.*, **20,** 129 (1932); P. Haas and T. G. Hill, *Ann. App. Biol.*, **7,** 352 (1921); P. Haas, *Biochem. J.*, **15,** 469 (1921); C. L. Butler, *Biochem. J.*, **28,** 759 (1934); T. Dillon and P. O'Colla, *Nature*, **145,** 749 (1940); G. Lunde, S. Lunde and A. Jakobson, *Fiskeri direktor Skrifter Ser. Haverundersk* (*Rept. Norweg. Fishery Marine Investigation*), **5,** No. 5, 21 (1938); E. G. V. Percival and J. Buchanan, *Nature*, **145,** 1020 (1940).

(116) E. G. Young and F. A. H. Rice, *J. Biol. Chem.*, **156,** 781 (1944); **164,** 35, (1946).

(117) V. C. Barry and T. Dillon, *Proc. Roy. Irish Acad.*, **50B,** 349 (1945).

(118) E. T. Dewar and E. G. V. Percival, *Nature*, **156,** 633 (1945).

(119) V. C. Barry and T. Dillon, *Nature*, **146,** 620 (1940).

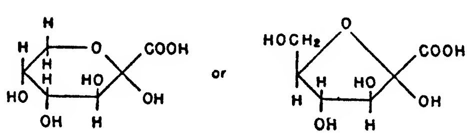
(120)(*a*) V. C. Barry, *Sci. Proc. Roy. Dublin Soc.*, **21,** 615 (1938); (*b*) *ibid.*, **22,** 59 (1939); (*c*) *ibid.*, **22,** 423 (1941); *ibid.*, **152,** 538 (1943); T. Dillon, *ibid.*, **155,** 546 (1945).

(121) C. Neuberg and H. Ohle, *Biochem. Z.*, **125,** 311 (1921); F. Fairbrother and H. Mastin, *J. Chem. Soc.*, **123,** 1412 (1923); W. F. Hoffman and R. A. Gortner, *J. Biol. Chem.*, **65,** 371 (1925); M. R. Butler, *Biochem. J.*, **28,** 759 (1934); M. Lüdtke, *Biochem. Z.*, **212,** 419 (1929); D. R. Nanji, F. J. Patton and A. R. Ling, *J. Soc. Chem. Ind.*, **44,** 253T (1925); N. W. Pirie, *Biochem. J.*, **30,** 369 (1936); E. G. V. Percival, J. Munro and J. C. Somerville, *Nature*, **139,** 512 (1937); S. Hands and S. Peat, *Chem. & Ind.*, 937 (1938); *Nature*, **142,** 797 (1938); E. G. V. Percival, J. C. Somerville and I. A. Forbes, *ibid.*, **142,** 797 (1938); I. A. Forbes and E. G. V. Percival, *J. Chem. Soc.*, 1844 (1939); E. G. V. Percival and T. H. Soutar, *ibid.*, 1475 (1940); R. B. Duff and E. G. V. Percival, *ibid.*, 830 (1941); W. G. M. Jones and S. Peat, *ibid.*, 225 (1942); W. E. Isaac, M. H. Finlayson and M. G. Simone, *Nature*, **151,** 532 (1943); E. G. V. Percival and J. C. Somerville, *J. Chem. Soc.*, 1615 (1937).

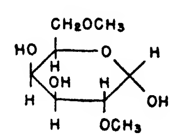
The most commonly encountered seaweed polysaccharides, excluding alginic acid, are agar and carragheen mucilage. The latter is obtained by aqueous extraction of Irish moss (*Chrondus* or *Chondrus crispus*), one of the red algae. They both have the property of giving a more or less mucilaginous or thick solution with water in the cold which after heating sets to a rigid gel.

D-Galactose has long been known to be the main component of the products of hydrolysis of carragheen, and Buchanan, Percival and Percival[122] found some 32% to be present as well as small quantities of D-glucose. Ketoses to the extent of about 20% also appear to be present.

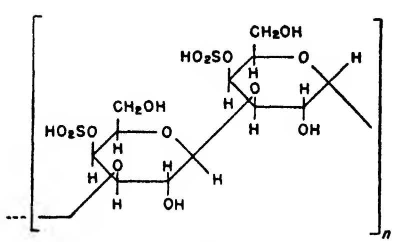
P. Haas and coworkers[108] had earlier shown that the mucilage was an ethereal sulfate and that it was a mixture, a result which was confirmed by E. G. V. Percival and coworkers[122] who demonstrated that extraction of the algae with cold water yielded a mucilage with physical properties differing slightly from those shown by material obtained by hot water extraction. The mucilage (LXIV) on acetolysis is degraded, all the sulfate and non-galactose components being removed during the operation; the only product which could be isolated was a galactan derivative.[115] Direct methylation of the polysaccharide showed that the ethereal sulfate grouping was not eliminated during this operation and that no more than 14.2% of methoxyl could be introduced into the molecule.[122] On boiling with dilute oxalic acid solution the methylated polysaccharide (LXV) was hydrolyzed with the simultaneous formation of sulfuric acid and reducing sugars which were then separated after conversion to their acetyl derivatives, by careful fractional distillation.



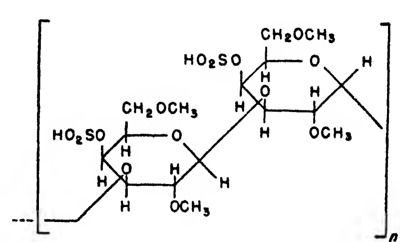
LXIII



LXVI



LXIV



LXV

(122) J. Buchanan, Elizabeth E. Percival and E. G. V. Percival, *J. Chem. Soc.*, 51 (1943).

The two main products identified were 2-methyl-D-galactose (XXXIV) and 2,6-dimethyl-D-galactose (LXVI).

The authors[122] believe that the sulfuric acid residue is located on C4 because of the great difficulty experienced in removing it with alkaline reagents, and that the linkage between the galactopyranose residues is between the hydroxyl groups on C1 and C3 of neighboring sugar units. It is considered unlikely that the sulfate residue is on C3 as it is known that such a sulfate linkage is readily hydrolyzed with the formation of a 3,6-anhydrogalactose residue. The resistance to methylation of the hydroxyl group on C6 is considered to be due to the steric effect of the sulfate group on C4. The polysaccharide was therefore postulated as being built up of 1,3-galactose linked residues similar to the galactan components in *Gigartina stellata*,[118] agar,[121] snail galactogen,[123] damson gum, gum arabic and other naturally occurring polysaccharides (see above).

This proposed structure LXIV is an oversimplification since 2-keto-D-gluconic acid (LXIII) has been identified among the products of hydrolysis of carragheen mucilage.[116] The presence of this material is probably, in part, responsible for the positive tests for ketose sugars obtained by earlier workers. A keto-hexonic acid may also be present in the galactan sulfate isolated from *Gigartina stellata.*

Agar is a gel-forming polysaccharide possessing properties similar to those of carragheen; the gel is extensively used for bacteriological culture purposes. From a hot solution a rigid gel is formed at 35–50°, which does not liquify on reheating until the temperature approaches 100°. The gel is susceptible to hydrolysis with very dilute acid and consequently the pH of acidic media for bacteriological purposes must be adjusted after sterilization.

Agar is extracted by hot water from various species of *Gelidium,* marine algae of the *Rhodophyceae.* The product may be purified by freezing the gel which is obtained on cooling the extract; on allowing it to thaw water, containing impurities, runs away leaving the purified product. By this means Barry, Dillon and also Percival obtained a product which contained traces only of sulfur,[121] indicating that only small quantities of ethereal sulfate were combined in the polysaccharide. It may be, however, that under the conditions of isolation (boiling the seaweed with water) some of the sulfate residues initially present in the polysaccharide were removed by hydrolysis.

Agar may be acetylated with pyridine and acetic anhydride and the resultant acetyl derivative readily converted into the corresponding methyl ether by reaction with sodium hydroxide and methyl sulfate;

(123) E. Baldwin and D. J. Bell, *J. Chem. Soc.*, 1461 (1938).

the maximum methoxyl content obtained in the methylated polysaccharide by this procedure was 31%. This figure indicates that some hydroxyl groups are protected from methylation in some manner since a fully methylated polyhexosan should contain 45.8% of methoxyl groups. On acidic hydrolysis of this methylated derivative Percival and Somerville[121] isolated 2,4,6-trimethyl-D-galactose (XIX), proving the presence of 1,3-linked D-galactopyranose residues in the molecule. Pirie[121] earlier had shown that acetolysis of agar gave in small yield heptaacetyl-D,L-galactose. The presence of an L-galactose derivative was confirmed by the work of Percival, Somerville and Forbes, and Hands and Peat[121] who isolated 2,4-dimethyl-3,6-anhydro-L-galactose (LXVII) from the further methylation of the products of hydrolysis derived from the methylated polysaccharide. The structure of this material was placed beyond doubt by the synthesis of the corresponding optical enantiomorph[124a, 124b] by methylation of the known methyl 3,6-anhydro-α-D-galactoside.[125] The mode of linkage of these sugars has been investigated by Percival and Thomson[126] and by Jones and Peat.[127] The former authors showed that on acetolysis of methylated agar followed by oxidation, a mixture of disaccharide esters could be obtained which on hydrolysis and suitable treatment yielded tetramethyl-D-galactopyranose anilide together with a mixture of methylated acids, amongst which 2,4,5,6-tetramethyl-D-galactonic acid (LXVIII) and 2,5-dimethyl-3,6-anhydro-L-galactonic acid (LXIX) were detected. The isolation of tetramethyl-D-galactopyranose anilide proves that D-galactose is united through its reducing group either to position 3 of a D-galactose residue (the only hydroxyl group in LXVIII) or to position 4 of a 3,6-anhydro-L-galactose residue (the only hydroxyl group in LXIX). These authors[126] were unable to detect any D-galactose end group present in the hydrolysis products of methylated agar. Jones and Peat[127] presented evidence that agar is a linear polysaccharide, the unit chain (repeating unit) of which is composed of nine residues of D-galactose combined by 1,3-glycosidic linkages (LXX). This chain is terminated at the reducing end by a residue of L-galactose, which is united with the remainder of the chain by a glycosidic linkage engaging C4 and not C3. The L-galactose residue, it is suggested, is esterified at C6 with sulfuric acid; removal of this sulfuric grouping results in the formation of a 3,6-anhydro-L-galactose derivative which is very unstable in the presence of weak acids,

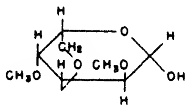
(124) (*a*) I. A. Forbes and E. G. V. Percival, *J. Chem. Soc.*, 1844 (1939); (*b*) W. N. Haworth, J. Jackson and F. Smith, *J. Chem. Soc.*, 620 (1940).

(125) H. Ohle and H. Thiel, *Ber.*, **66**, 525 (1933).

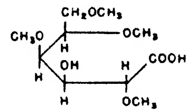
(126) E. G. V. Percival and T. G. H. Thomson, *J. Chem. Soc.*, 750 (1942).

(127) W. G. M. Jones and S. Peat, *J. Chem. Soc.*, 225 (1942).

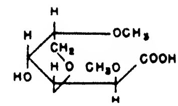
which hydrolyze the glycosidic linkage of this sugar with the resultant formation of LXX.



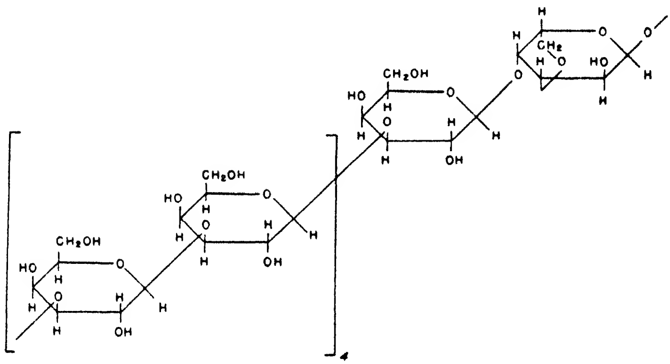
LXVII



LXVIII



LXIX



LXX

This conception of the structure of agar explains the isolation of L-galactose from it and also the separation of 2,4-dimethyl-3,6-anhydro-L-galactose and 2,5-dimethyl-3,6-anhydro-L-galactonic acid (LXIX) from methylated agar. These authors[127] have made the interesting suggestion that the L-galactose-6-sulfate derivative, substituted at C4, arises from a D-galactose 1-sulfate derivative substituted at C4, by an intramolecular oxidation-reduction change. Percival and Thomson, however, find it difficult to believe that this relatively simple structure (LXX) is the correct one for agar. They point out that the sulfate content of agar is too low to allow for the presence of one L-galactose 6-sulphate residue per nine D-galactose residues and that the methoxyl content of such a polysaccharide when fully methylated should be about 42%, whereas the maximum figure obtained was 35%. These authors[126] also found evidence for the presence of dimethyl galactose derivatives in the products of hydrolysis of methylated agar. No such derivative would occur in the products of hydrolysis of the methyl derivative of LXX. They come to the conclusion that the difficulty encountered in completely methylating agar may explain some of the discrepancies, such as the isolation of a dimethyl galactose derivative and the absence of an end group. Undoubtedly, some of the discrepancies in these results are due to the fact that the experimental work has been carried

out on commercial samples of unknown origin and unknown previous history.

The function of the sulfate residue in these polysaccharides is unknown but the suggestion has been made that just as starch is synthesised from D-glucose 1-phosphate by the action of phosphorylase, so the seaweed polysaccharides are formed from the appropriate sugar sulfate by reaction with a sulfatase.[128]

The action of periodic acid (a reagent diagnostic for α-glycols) on agar has been studied by V. C. Barry, who has shown that the polysaccharide consists mainly of 1,3-linked hexopyranose residues since this reagent is practically without effect on the polysaccharide.

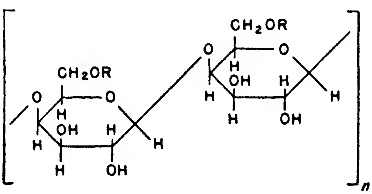
The galactan known as "Nori," isolated from *Porphyra laciniata*, resembles agar in that it contains L-galactose; D-mannose is also a component of the polysaccharide.[129]

The galactan derivative from *Irideae laminarioides* has been investigated in detail by Hassid.[107] This polysaccharide (LXXI; R = SO_2H) material is a sulfate ester, each galactose residue containing one ester sulfate grouping. On acetylation a diacetyl derivative is produced which on hydrolysis yields a sulfate-free galactan (LXXI; R = H) (compare the acetolysis of carragheen). On methylation this product is converted in the usual manner into a trimethyl ether, which yields on hydrolysis a trimethyl-D-galactose considered to be LIX. On the other hand, the polysaccharide LXXI on methylation yields a corresponding dimethyl derivative which still contains a sulfate residue and which on hydrolysis yields a dimethyl galactose. The proof of the identity of the trimethyl sugar rests on the following observations. No crystalline phenylosazone could be isolated on reaction of the sugar with phenylhydrazine and acetic acid and it was inferred that there was a methoxyl group on C2. On oxidation with nitric acid the acid derived from LIX by oxidation with bromine water gave a dimethoxy-glutaric acid derivative, isolated as its dimethyl ester ($[\alpha]_D + 41°$); this may have been an α,β (LXXII) or an α,γ-L-(*arabo*)-dimethoxy (LXXIII) derivative. In the first instance the product could have arisen from the oxidation of a 2,3,5- or a 2,3,6-trimethyl-D-galactose; in the second case the dimethyl ester would have been produced from the oxidation of 2,4,5- or 2,4,6-trimethyl-D-galactose. The optical rotation of the sugar (+129°) is characteristic of a pyranose sugar and the possible presence of a 2,3,5-trimethyl and 2,4,5-trimethyl-D-galactose derivative may therefore be eliminated. Hassid preferred to believe that the dimethoxy-glutaric acid derivative was LXXII, derived from the oxidation of 2,3,6-tri-

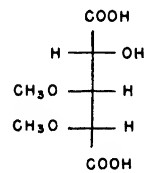
(128) C. S. Hanes, *Proc. Roy. Soc.*, **B128**, 421 (1940); **B129**, 174 (1940).

(129) K. Oshima and B. Tollens, *Ber.*, **34**, 1422 (1901).

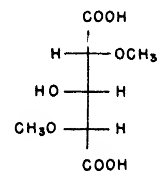
methyl-D-galactose as at that time 1,3-linked polysaccharides were unknown. It is very probable, however, that the sugar derivative was in fact the 2,4,6-trimethyl-D-galactose ([α]D *ca.* 110°) (XIX) which would give on oxidation the α,γ-dimethoxy derivative of L-(*arabo*)-glutaric acid (LXXIII), the dimethyl ester of which has [α]D + 41°.[12] The constitution of this polysaccharide would then fall into line with that of polysaccharides such as agar and carragheen, isolated from other seaweeds; the sulfate group may be located on either C4 or C6 but is most likely on C4 as it is known that a sulfate group on this position is stable to alkalies. A possible formula is given in LXIV.



LXXI

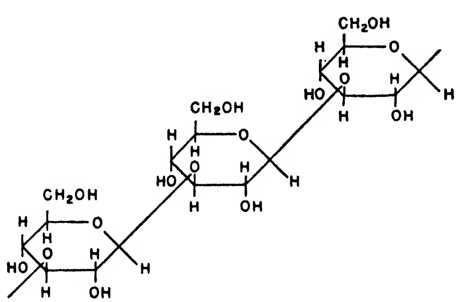


LXXII

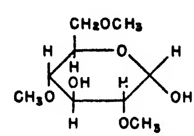


LXXIII

The polysaccharide laminarin, extracted from *Laminaria digitata*, has been shown by Barry to be composed of D-glucose units, some of which contain sulfate groups.[120a] By the normal procedure of acetylation and methylation, followed by hydrolysis of the methyl derivative, it was established that laminarin is a D-glucose type of polysaccharide the constituent members of which are joined by β-1,3-glycosidic linkages as in LXXIV. This follows from the identification of crystalline 2,4,6-trimethyl-D-glucose (LXXV) as the cleavage product of methylated laminarin and the low optical activity of the polysaccharide and its derivatives.[120b]



LXXIV



LXXV

6. *Conclusion*

From the above account of the chemistry of the mucilages it will be seen that very little is known of the more detailed structure of any of

these complex compounds and that much further work will be necessary before any generalizations can be made as to the constitution of these polysaccharides. The work of Doudoroff, Hassid and Barker,[130] however, has shown that one enzyme using different substrates is capable of synthesizing sugar disaccharides in which the sugars are joined together by a trehalose type of linkage in some cases and by a 1,3-linkage in another example. This enzyme, phosphorylase from *Pseudomonas saccharophila*, is capable of synthesizing D-glucosido-D-xyloketose from D-glucose-1-phosphate and D-ketoxylose, and also D-glucosido-3-L-arabinose from D-glucose 1-phosphate and L-arabinose, as well as sucrose and D-glucosido-L-sorbofuranose from D-glucose 1-phosphate and the appropriate substrate. It is possible, therefore, that comparatively few enzyme systems may be involved in putting together the complex mixture of sugars which are encountered in some of the plant mucilages and gums.

III. Tables of Derivatives of Pentoses, Desoxyhexoses and Hexoses Isolated from Plant Gums and Mucilages

In the following tables are to be found sugar derivatives which have been isolated in studies on plant gums and mucilages. Other references are included when they afford additional data for the further characterization of a compound.

The following abbreviations are used: W (water), M (methyl alcohol), E (ethyl alcohol), A (acetone), C (chloroform), P (pyridine), EA (ethyl acetate), and B (benzene).

(130) M. Doudoroff, W. Z. Hassid and H. A. Barker, *J. Biol. Chem.*, **168**, 725, 733 (1947); *cf.* A. Gottschalk, *Nature*, **160**, 113 (1947).

TABLE VIII
Derivatives of Pentoses

Compound	*Melting point °C.*	$[\alpha]_D$ *(solvent)*	*Reference*
L-Arabinose			
2-methyl			
3,4-monoacetone	116–118	+91.5 (C), +125 (M), +117.5 (A)	131
β-methylglycoside	63–65	+208 (M)	131
hydrate	46–47		131
3-methyl			
anilide	117		132*a*
phenylosazone	163		132*b*
2,3-dimethyl			
anilide	139		132*b*, 133
3,5-dimethyl			
phenylosazone	170		46
L-Arabonic Acid			
3-methyl			
lactone	78	−74 (W)	132*a*
amide	132		132*a*
2,3-dimethyl			
lactone	35	−38 → −25 (W)	132
amide	162	+17 (W)	133
2,4-dimethyl			
amide	158		12
2,5-dimethyl			
lactone	60	−60 (W)	12
amide	132	+38 (W)	31
phenylhydrazide	163		38, 31
3,5-dimethyl			
lactone	78	−43 (C)	46, 132*a*
		−84 (W)	47
amide	145	+10 (W)	47, 132*a*
2,3,4-trimethyl			
amide	156	+24.4 (W)	12
2,3,5-trimethyl			
lactone	33	−45 → −24 (W)	*cf.* 134, 12
amide	138	+16 (W), +21 (E)	12, 31, 36, 38, 46, 47
D-Xylose			
2-methyl	132–3	−24 → +36 (W)	135
2,3-dimethyl			
anilide	146	+185 (EA)	136
2,4-dimethyl	108	−30 → +22	137
anilide	170		137
2,3,4-trimethyl	91–2	+64 → +18 (W), +54 (C)	7, 33*a*, 138

TABLE VIII (*Continued*)

Compound	*Melting point °C.*	$[\alpha]_D$ (*solvent*)	*Reference*
D-Xylonic Acid			
2,3-dimethyl			
amide	133.5	+46 (W)	20, 139
phenylhydrazide	107–8	+30 (E)	136
p-bromophenyl-hydrazide	150–1		136
3,4-dimethyl			
lactone	68		7, 99
2,3,4-trimethyl			
lactone	56	−4 → +21 (W)	7
phenylhydrazide	138		140

(131) Mary A. Oldham and J. Honeyman, *J. Chem. Soc.*, 986 (1946).
(132) (*a*) E. L. Hirst, J. K. N. Jones and E. Williams, *J. Chem. Soc.*, 1062 (1947); (*b*) F. Smith, *ibid.*, 753 (1939).
(133) E. L. Hirst and J. K. N. Jones, *J. Chem. Soc.*, 1868 (1939).
(134) J. Avery, W. N. Haworth and E. L. Hirst, *J. Chem. Soc.*, 2317 (1927).
(135) G. J. Robertson and T. H. Speedie, *J. Chem. Soc.*, 825 (1934).
(136) H. A. Hampton, W. N. Haworth and E. L. Hirst, *J. Chem. Soc.*, 1739 (1929).
(137) C. C. Barker, E. L. Hirst and J. K. N. Jones, *J. Chem. Soc.*, 784 (1946).
(138) F. P. Phelps and C. B. Purves, *J. Am. Chem. Soc.*, **51**, 2443 (1929).
(139) R. A. S. Bywater, W. N. Haworth, E. L. Hirst and S. Peat, *J. Chem. Soc*, 1983 (1937).
(140) W. N. Haworth and C. W. Long, *J. Chem. Soc.*, 345 (1929)

TABLE IX

Derivatives of 6-Desoxyhexoses (Methylpentoses)

Compound	*Melting point °C.*	$[\alpha]_D$ (*solvent*)	*Reference*
L-Fucose			
2,3,4-trimethyl	36–37	$-184 \rightarrow -128$ (W)	56
hydrate	65	$-169 \rightarrow -118$ (W)	56
anilide	133–134	-77 (E)	55
α-glycoside	97–98	-209 (W)	56
β-glycoside	101.5–102.5	-21 (W)	56
L-Fuconic Acid			
2,3,4-trimethyl			
amide	102–103	-35 (W)	55
L-Rhamnose			
4-methyl	125–126	$+13$ (M)	141
phenylosazone	162–163	$+26.0 \rightarrow +14$ (P–E)	141
3,4-dimethyl	102–103	$0 \rightarrow +18.6$	9, 142
	91–2		105
2,3,4-trimethyl			
anilide	111		31
L-Rhamnonic Acid			
4-methyl			
lactone	82	$-141 \rightarrow -115$ (W)	101*b*
3,4-dimethyl			
lactone	76–78	$-150 \rightarrow -116$ (W)	8, 9
amide	152–155		8, 105
2,3,4-trimethyl			
lactone	40–41	$-130 \rightarrow -78$ (W)	143
phenylhydrazide	177		31, 143

(141) P. A. Levene and J. Compton, *J. Biol. Chem.*, **114**, 9 (1936).
(142) R. S. Tipson and P. A. Levene, *J. Biol. Chem.*, **130**, 235 (1939).
(143) J. Avery and E. L. Hirst, *J. Chem. Soc.*, 2466 (1929).

TABLE X
Derivatives of Hexoses

Compound	*Melting point °C.*	$[\alpha]_D$ *(solvent)*	*Reference*
D-Galactose			
2-methyl	145–148	+52 → +83 (W)	144, 145
anilide	165		145
β-methylglycoside	131–132	+1.7 (W)	44, 122
4-methyl	207	+62 → +92 (W)	36
phenylosazone	150		33
anilide	168		36
6-methyl	128	+114 (W)	146
β-methylglycoside	114–115	±0 (W)	144
phenylosazone	204–205	+135 (P)	122, 146
2,4-dimethyl			
hydrate	103	+122 → +86 (W)	33, 36
α-methylglycoside	105	+142 (W)	33, 123
β-methylglycoside	166	±0.0 (W)	36, 46
anilide	216		33, 36
2,6-dimethyl	128–130	+47 → +87.5 (W)	144
	120	+48 → +87	118
β-methylglycoside	46.5	−23 (C)	118
anilide	121–122	+15 (E)	118
4,6-dimethyl			
phenylosazone	158	−23 (E)	167
2,3,4-trimethyl	79	+154 → +114 (W)	33
anilide	167		33
	170		33*a*, 48
2,4,6-trimethyl	105	+124 → +93 (W)	167
anilide	179	−92 → +38 (A)	33*a*, 36, 48
α-methylglycoside	73–74	+164 (W)	167
β-methylglycoside	102	+18 (M)	36
hydrate	83–85		36
2,3,4,6-tetramethyl	75	+149 → +117 (W)	33, 148
anilide	192	−83 → +41 (A)	12, 69, 72, 147
	197		33*a*
β-methylglycoside	48.5	+19.6 (W)	14, 148
		−24 (E)	148
L-Galactose			
2,3,4,6-tetramethyl			
anilide	197	+70 (A)	126

(For the properties of the methyl derivatives of 3,6-anhydro-L-galactose see *Advances in Carbohydrate Chemistry*, **2**, **77** (1946).)

D-Galactonic Acid			
2,4-dimethyl			
lactone	113	+162 → +52.6 (W)	33
amide	167	+59 (W)	33, 36
phenylhydrazide	183		33

TABLE X (*Continued*)

Compound	*Melting point °C.*	$[\alpha]_D$ (*solvent*)	*Reference*
2,6-dimethyl			
amide	154–155	+46 (W)	118
phenylhydrazide	140	−45 (E)	118, 149
2,3,4-trimethyl			
amide	165	+32 (W)	33
phenylhydrazide	176	+32 (E)	17, 33
2,3,6-trimethyl			
lactone	99	−40 → −28 (W)	101*b*, 150
	101	−44 → −29 (W)	17
amide	135		101*b*
phenylhydrazide	175		17
2,4,6-trimethyl			
amide	166	+73 (W)	36
2,3,4,6-tetramethyl	84	+22.6 (W)	151
amide	121–122	+36 (A)	12, 152
phenylhydrazide	135–137		12, 153
2,3,4,5,6-pentamethyl			
methyl ester	46	+20 (W)	126
D-Galacturonic Acid			
α-methylpyruronoside			
methyl ester	148		154*a*
hydrate	110–112	+131 (W)	154*b*
amide	225–226	+127 (W)	154*a*
2,3-dimethyl			
β-methylpyruronoside	111	−11 (W)	155*b*
β-methylfururonoside			
amide	124	−151 (W)	155*a*
2,3,4-trimethyl			
α-methylpyruronoside			
methyl ester	72	+170 (W), +149 (A)	9, 155*a*
		+142 (C)	154*a*
amide	153	+121.5 (C)	156
β-methylpyruronoside			
methyl ester	102	−21 (M), −7 (W)	54
2,3,5-trimethyl			
β-methylfururonoside			
methyl ester	42	−123 (M)	155*a*
amide	106	−151 (W)	155*a*
D-Galactosaccharic Acid			
2,3-dimethyl			
6-methyl-1,4-lactone	92	−56 → −4 (W)	155*a*
diamide	228		155*a*
bis(methylamide)	184	−7.5 (W)	155*a*
2,3,5-trimethyl			
6-methyl-1,4-lactone	62	−83 (W)	155*a*
diamide	255		155*a*

TABLE X (*Continued*)

Compound	*Melting point °C.*	[α]D (*solvent*)	*Reference*
2,4-dimethyl			
1-methyl-3,6-lactone	111	+120 (W)	33
diamide	229	+30 (W)	33
bismethylamide	214	+27 (W)	33
2,4,5-trimethyl			
1-methyl-3,6-lactone	64	+85 (W)	33
diamide	225(d)		33
bismethylamide	232(d)	+23 (W)	33
2,3,4-trimethyl	101	+42 (A)	154*a*
1,6-dimethyl	103	+36 (W)	14, 33
diamide	273(d)		33
bismethylamide	205	+7.5	33
	207	+12.6 (M)	154*a*
6-methyl-1-amide	156	+34 (W)	33
2,3,4,5-tetramethyl			
1,6-dimethyl	109	inactive	33
diamide	276		157
D-Glucose			
2,4,6-trimethyl	113–114	+99 → +74 (W)	120*b*
D-Glucuronic Acid			
2,3-dimethyl			
methylglucuronoside			
methyl ester			
p-nitrobenzoate	157		31
phenylhydrazide	225–227		31
2,3,4-trimethyl			
β-methylglucuronoside	133	−38 (W)	14
		−63 (C)	14
amide	193	−47 (W)	33
α-methylglucuronoside			
amide	183	+138 (W)	33
6-(β-2,3,4-Trimethyl-D-glucuronosyl)-2,3,4-trimethyl-D-galactose			
β-methylglycoside			
methyl ester	94	−21 (W)	14, 15
amide	196	−18 (W)	15
D-Glucosaccharic Acid			
2,3-dimethyl			
amide	156	+28 (W)	31
6-methyl-1,4-lactone	101	+14 → +28 (W)	31, 33*a*
2,3,4-trimethyl			
6-methyl-1,5-lactone	107	+102 → +52 (C)	18
		+103 → +32 (W)	33
		+102 → +52 (M)	33, 46
		+100 (E)	158
		+175 (B)	2, 158

TABLE X (*Continued*)

Compound	*Melting point °C.*	$[\alpha]_D$ (*solvent*)	*Reference*
2,3,5-trimethyl			
6-methyl-1,4-lactone	78	−10 (W)	31
diamide	213	+18 (W)	159
D-Mannose			
α-methylglycoside	190	+79 (W)	160
3,4-dimethyl			
hydrate	107–109	+3 (W)	69, 161
2,3,6-trimethyl			
anilide	127–128	−155 → −39 (M)	70, 72, 162
3,4,6-trimethyl	101–102	+22 → +8.2 (W), +36 (M)	166*a*
anilide	143	+154.5 → −55.5 (M)	163
2,3,4,6-tetramethyl	51.5	+2.4 (W), +23 (C), +27.5 (M)	164
anilide	143	−88 → −8 (M)	72, 147, 162, 163
α-methylglycoside	40	43 (W), +70.5 (M), +75.5 (E)	165
β-methylglycoside	38	−80 (W), −79 (M), −87 (C), −72 (B), −82 (E)	166*b*
D-Mannonic Acid			
2,3-dimethyl			
lactone	111		70, 72
phenylhydrazide	168		70, 72
3,4-dimethyl			
lactone	157–158	+174 → +129 (W)	161
amide	140	+22 (W)	69
2,3,6-trimethyl			
lactone	85	+65 (W)	70, 72, 162
phenylhydrazide	135	−21 (W)	70, 72, 162
amide	125	−16 (W)	72
3,4,6-trimethyl			
lactone	97	+169 → +133 (W)	163
phenylhydrazide	139		166*a*
amide	141	+25 (W)	2, 163

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(151) W. N. Haworth, D. A. Ruell and G. C. Westgarth, *J. Chem. Soc.*, **125**, 2468 (1924).

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(155) (*a*) Sybil P. Luckett and F. Smith, *J. Chem. Soc.*, 1106 (1940); (*b*) *ibid.*, 1506 (1940).

(156) P. A. Levene and L. C. Kreider, *J. Biol. Chem.*, **121**, 155 (1937).

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(162) F. Klages, *Ann.*, **509**, 159 (1934); **512**, 185 (1934).

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(164) M. L. Wolfrom and W. L. Lewis, *J. Am. Chem. Soc.*, **50**, 837 (1928); R. D. Greene and W. L. Lewis, *ibid.*, **50**, 2813 (1928).

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THE UTILIZATION OF SUCROSE

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Contents

I. Introduction

Projects for the exploration of the ways in which sucrose may be used as a source of new industrial materials and intermediates have recently been started in the United States and in Great Britain, and it is appropriate that these volumes should contain an account from time to time of developments in this field.

The problem of the utilization of sucrose in ways other than directly as a food has engaged the minds of chemists and others for many years past, but the earlier efforts were not made as a part of any organized program. Two organizations, the Sugar Research Foundation Inc. in America and the Colonial Products Research Council in Great Britain, have been formed, and the development of the industrial use of cane sugar is included in each of their programs.

It might be argued that there is little purpose in exploring new outlets for cane sugar at a time when there is a world shortage of it. This, however, is a short-sighted view. Sugar cane or sugar beet can be easily cultivated and the sugar efficiently extracted and, since sugar factories have for the most part escaped damage during the war, in a very few years sugar production will reach pre-1939 proportions. We shall then be faced with the old problem of finding a market for the surplus sugar. It has been calculated that in the British West Indies alone production

could be practically trebled in a relatively short period of time if occasion demanded, to give something like a million tons annually. The livelihood of many colonial peoples depends on sugar cultivation which, because of overproduction, was artificially curtailed by international agreement before the second world war.

TABLE I[1]

Principal sugar-producing countries	*Metric tons beet sugar*	*Metric tons cane sugar*
United States of America	1,528,600	494,300
U.S.S.R.	2,152,400	—
Germany	1,911,200	—
Belgium	174,700	—
Denmark	767,000	—
France	164,300	—
Italy	369,800	—
Netherlands	191,300	—
Poland	491,300	—
Great Britain	302,400	—
Sweden	263,100	--
Czechoslovakia	460,900	—
British West Indies	—	350,000
Cuba	—	2,887,900
Trinidad and Tobago	—	130,500
British Guiana	—	192,300
Mexico	—	352,500
Puerto Rico	—	731,000
Dominican Republic	—	409,700
Mauritius	—	321,300
Union of South Africa	—	474,200
Argentina	—	465,600
Brazil	—	1,100,400
Peru	—	403,500
Formosa	—	1,527,300
India	—	2,060,000
Philippines	—	957,700
Australia	—	783,300
Hawaii	—	843,000
Netherlands Indies (Java)	—	1,562,500

The size of the sugar industry in pre-war years may be gauged from the production figures given in Table I and could be greatly extended if the demand were to increase.

(1) The figures given are taken from the Year Book of the League of Nations for 1938–1939 and have been reduced to metric tons of refined sugar using the official conversion factors, since in some cases tons of raw sugar only were recorded.

Economically sugar cane is the better source of sucrose; in Florida the yield of sugar per acre of cane grown is reported to be 3 tons whereas only 2.15 tons of beet sugar is obtained per acre in the Netherlands. It would seem that those regions which cultivate sugar cane could most easily expand the industry, and in many tropical countries such industrial expansion is becoming an urgent necessity. The beet-growing countries of more temperate climate are already highly industrialized.

The project may be supported on other grounds. The world's source of utilizable carbon is now mainly lodged in coal and oil. These are capital commodities which are a diminishing asset, and as they become exhausted fresh deposits must be sought; the mining of coal will become increasingly difficult as deeper shafts are sunk to reach the new deposits. On the other hand the plant world provides a source of carbon which is perennially renewed. These substances we obtain in the form of annual revenue, garnered so long as the earth remains fertile.

In this article attention will be directed mainly toward the more modern work on the chemical transformations of sucrose and the substances that can easily be obtained from it.

II. By-products of the Sugar Industry

Sugar cane, as cut, consists of the cane itself, the leaves and the "tops." At present the "tops," which contain on a dry basis about 44% carbohydrate, 7% protein, 32% fiber, 2% fats and 10% ash, are turned back into the ground. This procedure may, however, be wasteful. Fertilizers must be used, but it might be possible to extract other products from the cane tops before they are used for this purpose. To give one example, the "tops" may be fermented to yield alcohol. Lonkar and Rao[2] have outlined a process whereby dry leaves ("trash") can be converted into paperboard or wrapping paper, a process which could be applied on a scale such that a factory dealing with 9,000 tons of "trash" annually could turn out 3,000 tons of paperboard.

Some 25% of the cane consists of a fibrous material known as bagasse and this is perhaps the most important by-product of the cane sugar industry. At present the bagasse produced at most sugar factories is used as a fuel for the boilers, since there is usually a shortage of fuel in countries growing cane. The calorific value of bagasse is high, namely 4,765 cals. per gram as determined by Hessey[3] for Queensland bagasse. In this connection it is interesting to note that bagasse can be made into briquettes by first coking it and then mixing with molasses.[4]

(2) K. V. Lonkar and S. N. G. Rao, *Intern. Sugar J.*, **46**, 270 (1944).

(3) R. W. G. Hessey, *Intern. Sugar J.*, **40**, 43 (1938).

(4) P. M. D. de Haer, F. M. van Lawick and P. K. C. van Wal, *Arch. Suikerind. Nederland en Ned. Indië*, **1**, 631 (1941).

The bagasse of certain areas is used in making structural board, a product which has good heat and sound-insulating properties and is very strong and light. Since bagasse contains about 50% of its weight of cellulose, the problem of the economic extraction of this material has received much attention.[5] A good cellulose is claimed to be produced in Cuba using the de la Roza process, in which bagasse is treated with sulfur dioxide before alkali treatment.[6] This product might find application for any of the purposes to which commercial cellulose is put.

When bagasse is burned the ash remaining is of such a composition that it can be used in the manufacture of bottle glass.[7]

Bagasse in admixture with molasses has also been suggested as a cattle food.[8] Bagasse, because of its pentosan content, may also be used as a source of furfural.[9,10]

The conversion of bagasse into resinous materials has from time to time been recorded. Thus a phenolic resin has been obtained by heating bagasse with phenol and hydrochloric acid, but the product was reported as being not so strong as Bakelite.[11] Molding powders have also been obtained from the lignin and cellulosic contents of bagasse.[12,13]

Sugar cane wax is an interesting by-product of the sugar industry and one which has for the most part been neglected. It occurs in the so-called "factory mud," which is usually discarded. It has been shown, however, that extraction of the wax is possible and that the product can be resolved into a fatty substance and a wax proper. The mud is extracted with benzene and the crude wax resolved into a pure wax and a fatty portion which is soluble in cold acetone.[14] Indian workers have preferred to use an initial extraction with petroleum ether and to purify the extract by treatment with nitric acid.[15] Alternatively the wax may be extracted directly from the expressed juice before the latter is processed for sucrose manufacture. The wax is of good quality approaching that

(5) S. I. Aronovsky and D. F. G. Lynch, *Ind. Eng. Chem.*, **30**, 790 (1938).

(6) J. J. de la Roza, *Intern. Sugar J.*, **51**, 364 (1939).

(7) S. del Mundo, *Philippine J. Sci.*, **60**, 125 (1936).

(8) A. R. Lamp, *Intern. Sugar J.*, **39**, 323 (1937).

(9) H. Nisio and S. Aoki, *Rept. Gov. Sugar Expt. Sta. Tainan Formosa*, No. 7,231 (1940).

(10) H. J. Brownlee and B. C. L. Summers, *Mfg. Chemist*, **16**, 424 (1945).

(11) T. Tatuno, F. Nisio, S. Aoki and K. Yamakuzi, *Bull. Agr. Chem. Soc. Japan*, **17**, 181 (1941); *Chem. Abstracts*, **36**, 6033 (1942).

(12) Anonymous, *Intern. Sugar J.*, **51**, 368 (1939).

(13) T. R. McElhinney, T. F. Clark and D. F. G. Lynch, *Modern Plastics*, **16**, 42, 76 (1939).

(14) R. T. Balch, U. S. Pat. 2,381,420 (1945).

(15) K. Aswath, N. Rao and G. N. Gupta, *Proc. 10th Ann. Convention Sugar Tech. Assoc. India.*, Pt. **2**, 79 (1941); *Chem. Abstracts*, **37**, 5268 (1943).

of Carnauba wax and gives a high polish, especially to leather goods. One firm isolating sugar cane wax reports that one ton of pure wax is obtained for every 1,000 tons of cane processed.

The disposal of sugar beet pulp does not offer any very serious problem, since it can readily be dried and used as a cattle food and it is probable that all the available supplies are absorbed in this way. The utilization of molasses is discussed separately in Section VII.

III. Conversion of Sucrose into Other Products by Chemical Methods

1. *Oxidation Products*

No oxidation products of sucrose other than those produced by degradation have been described. Oxidation of sucrose with periodic acid has been carried out,[16] but neither the method nor the products need to be considered for the present purpose.

Sucrose can be completely oxidized to carbon dioxide, a reaction which takes place in the presence of air and an alkaline medium and which is rightly blamed for losses which occur during manufacture. Nitric acid oxidation of sucrose takes place with formation of a variety of products the character of which depends on the conditions used. Oxalic acid is produced in 70% of the theoretical yield, when the reaction is catalyzed by the presence of ammonium vanadate.[17] Brooks[18] recommends the addition of 0.1% of ferric ion for the production of high yields of oxalic acid. Other workers have found nitric acid in the presence of vanadium pentoxide and molybdenum trioxide to be the most efficacious oxidizing agent.[19] Nordenstein, Pfannenstiel and Spengler[20] have described the production of oxalic acid by air oxidation of sucrose in alkaline solution.

Although many workers have been interested in the production of oxalic acid from sucrose, it is probably true that sucrose cannot compete with other raw materials as a source of this acid. For example, sawdust has been found to be an excellent source of oxalic acid. It is possible, however, to arrest the oxidation of sucrose before the oxalic acid stage is reached and the much more valuable tartaric acid has been obtained in this way. Tartaric acid, at the moment manufactured from waste

(16) P. Fleury and J. Courtois, *Compt. rend.*, **214**, 366 (1942); *Bull. soc. chim.*, **10**, 245 (1943).

(17) W. Dominik and J. Janczak, *Roczniki Chem.*, **14**, 141 (1934).

(18) M. J. Brooks (to General Chemical Co.), U. S. Pat. 2,322,915 (1944).

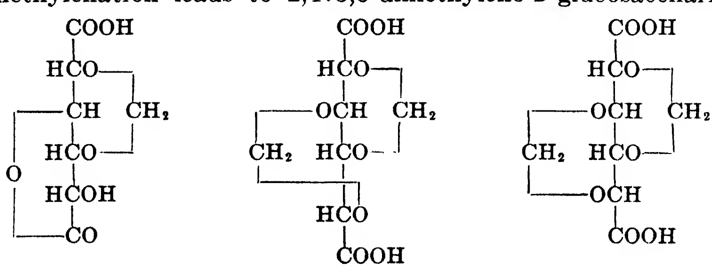
(19) Teng-Han Tang and F. C. Kao, *J. Chem. Eng. China*, **6**, 32 (1939); *Chem. Abstracts*, **35**, 5466 (1941).

(20) L. Nordström, A. Pfannenstiel and O. Spengler, *Z. Wirtschaftsgruppe Zuckerind.*, **89**, 171 (1939).

products of the wine industry, is a high-priced commodity and its production from sucrose would be advantageous. Soltzberg[21] oxidized sucrose with 70% nitric acid and a vanadium catalyst and obtained up to 42% yield of tartaric acid and 44% of oxalic acid. These new methods of obtaining tartaric acid may well compete with the older sources and, in addition, increase the availability of tartaric acid, uses for which already abound.

When the oxidation is carried out under mild conditions, the glucose portion of the sucrose molecule is converted into glucosaccharic acid, a yield[22] of 28% (calculated on sucrose) being reported. Braun and Breivik[23] have recently described a process for separating DL-, L- and *meso*-tartaric acids and D-glucosaccharic acid from such mixtures as may be obtained from the oxidation of sucrose. Air oxidation of sucrose in an alkaline medium ultimately gives oxalic acid but, if the reaction is discontinued before this stage is reached, high yields of D-arabonic acid can be obtained. This is an important product because it is convertible into the D-ribose needed for the manufacture of riboflavin.

D-Glucosaccharic acid might be regarded as a source of useful products but little success has as yet been achieved in this direction. Pyrrole has been obtained from D-glucosaccharic acid by the method used by Goldschmidt[24] with diammonium mucate. Fugita, Maschima and Hachihama[25] obtained pyrrole in 45% yield by heating glucosaccharic acid in glycerol with ammonia. Methylene derivatives of D-glucosaccharic acid are readily prepared; a monomethylene derivative was first isolated by Henneberg and Tollens[26] in 1896 and was proved to be 2,4-monomethylene 3,6-D-glucosaccharic lactone (I) by Jones and Wiggins.[27] Further methylenation leads to 2,4:3,5-dimethylene-D-glucosaccharic acid



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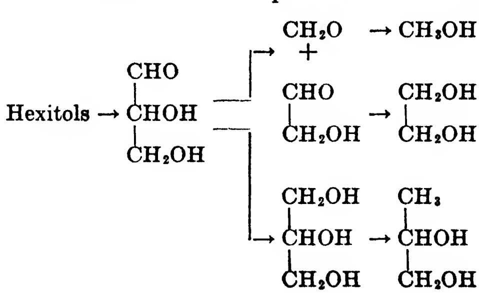
(21) S. Soltzberg (to Atlas Powder Co.), U. S. Pat. 2,380, 196 (1945).
(22) Y. Hachihama and H. Fugita, *J. Soc. Chem. Ind. Japan*, **38**, 744 (1935).
(23) G. Braun and O. N. Breivik, U. S. Pat. 2,382,288 (1945).
(24) M. Goldschmidt, *Z. Chem.*, **3**, 280 (1867).
(25) H. Fugita, T. Maschima and Y. Hachihama, *J. Soc. Chem. Ind. Japan*, **41**, 63 (1938); *Chem. Abstracts*, **32**, 6902 (1938).
(26) W. Henneberg and B. Tollens, *Ann.*, **292**, 40 (1896).
(27) W. G. M. Jones and L. F. Wiggins, *J. Chem. Soc.*, 364 (1944).

(II), which readily undergoes epimerization to form 2,4:3,5-dimethylene-L-idosaccharic acid (III).[28] Attempts have been made to utilize these acids as components of nylon-like polyamides, but these have met so far with little success.

2. *Reduction Products*

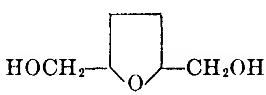
The hydrogenolysis of sucrose has been the subject of much study and several valuable products have been obtained. Sucrose readily undergoes hydrolysis in the presence of very small amounts of acids. The mixture of hydrolysis products, D-glucose and D-fructose, has been reduced catalytically to give D-mannitol and D-sorbitol so that the product consists of approximately two thirds of sorbitol and one third of mannitol. Alternatively, sucrose, without preliminary inversion, may be hydrogenated to give the same products. When the reaction is carried out at higher temperatures alcohols of smaller molecular weight are produced. Zartman and Adkins[29] carried out the hydrogenation of sucrose over copper–chromium oxide at 250°C. and 300 atmospheres in alcoholic solution and obtained a mixture of glycols containing principally propylene glycol. A better yield of this substance was obtained when D-glucose was used as the starting material, a fact suggesting that inversion of sucrose did not precede the degradation.

Weidenhagen and Wegner[30] found that acetol, CH_3COCH_2OH, was produced under certain conditions in this hydrogenation reaction and that ethylene glycol, propylene glycol and glycerol were also formed. Yosikawa and Hanai[31] described the hydrogenation of sucrose at 160° to 180°C. and 80 to 300 atmospheres over nickel or nickel–iron catalysts; hexitols are first formed and are then degraded to propylene glycol, ethylene glycol, glycerol and monohydric alcohols. The following scheme is proposed to account for the production of these alcohols:

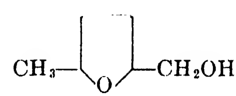


(28) W. N. Haworth, W. G. M. Jones, M. Stacey and L. F. Wiggins, *J. Chem. Soc.*, 61 (1944).
(29) W. H. Zartman and H. Adkins, *J. Am. Chem. Soc.*, **55**, 4559 (1933).
(30) R. Weidenhagen and H. Wegner, *Listy Cukrovar.*, **57**, 185 (1939).
(31) K. Yosikawa and S. Hanai, *Bull. Inst. Phys. Chem. Research (Tokyo)*, **17**, 1262 (1940); *Chem. Abstracts*, **34**, 2796 (1940).

In 1943, Natta, Rigamonti and Beati[32] published a detailed investigation of the hydrogenation of carbohydrates. D-Glucose was hydrogenated under a variety of conditions and glycerol, methylglycerol and ethylene glycol were obtained in the ratio 2:1:1. Although methylglycerol was produced only when nickel catalysts were used, with copper catalysts glycerol, propylene glycol, isopropyl alcohol, a small amount of ethylene glycol and still smaller amounts of methylpentitols were obtained. Lenth and Du Puis[33] investigated the possibility of obtaining glycerol from carbohydrates, including sucrose. The hydrogenation of sucrose in methanol solution at 235–245°C. and at 100 atmospheres pressure in the presence of a copper–aluminum catalyst and 0.4% of soda ash yielded 64.6% of the weight of sucrose as distillable polyhydric alcohols including glycerol (25% of the weight of sucrose) and propylene glycol (40%). These workers also isolated certain tetrahydrofuran derivatives including what were believed to be tetrahydrofuran 2,5-dicarbinol (IV), 5-methyltetrahydrofurfuryl alcohol (V) and α-methyl-4-hydroxytetrahydrofurfuryl alcohol (VI).



IV



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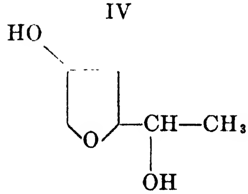
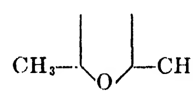


VI



VII

The writer and his coworkers have also studied the hydrogenation of sucrose, and it may be opportune to mention our ideas on the mechanism of the reaction at this juncture. When sucrose is hydrogenated in aqueous solution over Raney nickel, at about 120° to 130°, inversion of the sucrose begins and thereafter two distinct reactions can, and usually do, take place. One of these is the hydrogenation of the hexoses to hexitols. The other is a degradation of the hexoses to hydroxymethylfurfural (see below). If the rate of heating of the sucrose solution is very slow the second reaction is minimized, but if the heating is rapid this reaction occurs to such an extent that its by-product, humin material, de-activates the catalyst and the hydrogenation is arrested.

(32) G. Natta, R. Rigamonti and E. Beati, *Ber.*, **76**, 641 (1943).
(33) C. W. Lenth and R. N. Du Puis, *Ind. Eng. Chem.*, **37**, 152 (1945).

Normally, however, the reaction involving the formation of hydroxymethylfurfural proceeds less readily than does the hydrogenation of glucose and fructose to mannitol and sorbitol, but its occurrence is detected by the fact that tetrahydrofuran derivatives have been isolated from the hydrogenation products. Thus, we have isolated tetrahydrofuran 2,5-dicarbinol (identified as its ditosyl derivative), 5-methyltetrahydrofurfuryl alcohol and 2,5-dimethyltetrahydrofuran (VII) together with hydrogenolysis products of these compounds.

There are thus three main products of the hydrogenation of sucrose, namely, mannitol, sorbitol and the mixture of glycols obtained by high-temperature cracking. The mixed glycols could be used in place of pure ethylene glycol as an antifreeze material, and the glycerol constituent might be separated and used to augment the present supplies. Apparently this high-temperature cracking of sugar was carried out on a large scale by the I. G. Farbenindustrie A.-G. at Hoechst during the war. Mannitol and sorbitol are probably the most important of the hydrogenation products of sucrose, and, owing to the ease with which mannitol crystallizes, the two hexitols can be readily separated.

Sorbitol is of importance as a source of L-sorbose (VIII) for the synthesis of vitamin C. In addition, sorbitol has been used as a substitute for glycerol, as a humidifying agent in confectionery, for tobacco

CH_2OH
HCOH
HOCH
HCOH
CO
CH_2OH
VIII

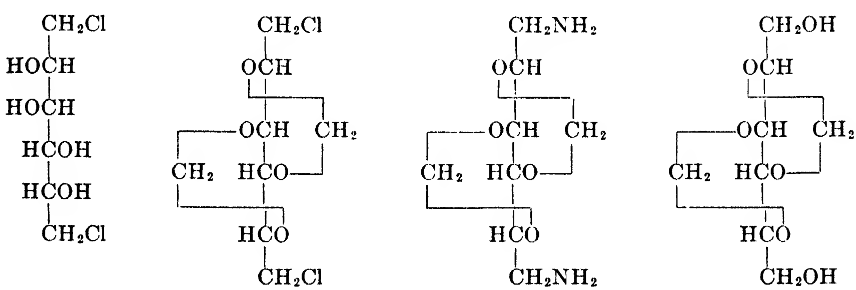
and leather processing and in sizings for textiles and paper. It has been used as a sugar substitute for diabetics. Sorbitol may also be used for making resins and the literature on this subject has been reviewed recently by Long.[34]

D-Mannitol is not only a product of the hydrogenation of sucrose or of D-glucose[35] but it is also a by-product of the fermentation of sugar to lactic acid and other special fermentations and is a by-product in the extraction of alginic acid from seaweeds. It is found in the sugar cane

(34) L. Long, "Sugar and Sugar By-Products in the Plastics Industry"; Rept. No. 1, Sugar Research Foundation Inc., New York (1945).
(35) H. J. Creighton, *Trans. Electrochem. Soc.*, **75**, 289 (1939).

itself. Mannitol was nitrated as early as 1840 by Sobrero, and the hexanitrate is of importance in that it is a safer detonator than mercury fulminate and is in fact used for this purpose. Several medicinal uses have been suggested for mannitol and its derivatives. The hexanitrate is a vasodilator and may be used in heart disorders.[36] Mannitol itself has been used as a mild laxative for children. It is also useful in making inoculants of nitrogen-fixing bacteria for soil improvement, since it possesses the advantage over other carbohydrates that no gas is formed during the growth of these bacteria. The gallates[37] of both mannitol and sorbitol have been prepared and it is a curious fact that only the gallate of mannitol shows good tannage.

The chemistry of mannitol and sorbitol has been studied with a view to finding further uses for these alcohols and in this connection might be mentioned an attempt, though unsuccessful, to make a polyamide intermediate from mannitol.[38] Mannitol was converted through the 1,6-dichloro derivative (IX) into 1,6-dichloro-dimethylene-mannitol (X). This product on treatment with ammonia gave 1,6-diamino-dimethylene-mannitol (XI), which, however, did not give oriented fibers after polymerization with adipic acid. Ester polymers were also made from 2,4:3,5-dimethylene-mannitol (XII), which was in turn prepared from 1,6-dibenzoylmannitol, but the products were not of immediate value.[39]

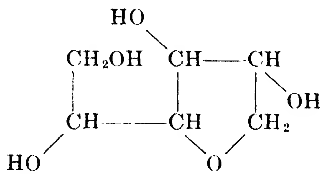


IX X XI XII

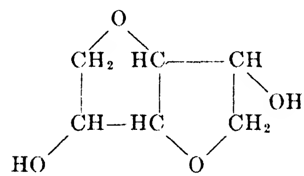
A development of some promise lies in the dehydration of mannitol and sorbitol to give anhydro compounds. Mannitol can be dehydrated to give 1,4-anhydro-mannitol (XIII) (mannitan) and isomannide, shown to be 1,4:3,6-dianhydro-mannitol (XIV) by Wiggins.[40] Similarly,

(36) C. J. Carr and J. C. Krantz, Jr., *Advances in Carbohydrate Chem.*, **1**, 175 (1945).
(37) A. Russell and W. G. Tebbens, *J. Am. Chem. Soc.*, **64**, 2274 (1942).
(38) W. N. Haworth, R. L. Heath and L. F. Wiggins, *J. Chem. Soc.*, 155 (1944).
(39) W. N. Haworth and L. F. Wiggins, *J. Chem. Soc.*, 58 (1944).
(40) L. F. Wiggins, *J. Chem. Soc.*, 4 (1945).

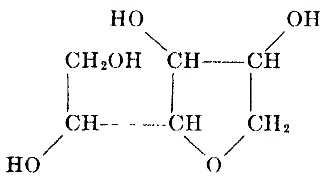
sorbitol can be dehydrated stepwise to sorbitan, shown to be a 1,4-anhydro-sorbitol (XV) by Hockett[41] and to isosorbide, which has been proved to be 1,4:3,6-dianhydro-sorbitol (XVI).[42,43] The dehydration of sorbitol proceeds easily and the product is obtained in about 70% yield; that of mannitol, however, is more difficult and smaller yields are obtained. A mixture of the dianhydro compounds of mannitol and



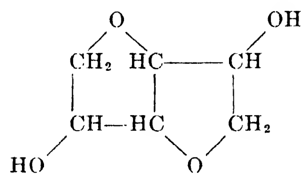
XIII



XIV

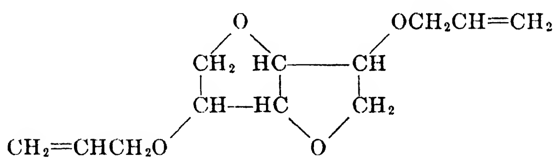


XV

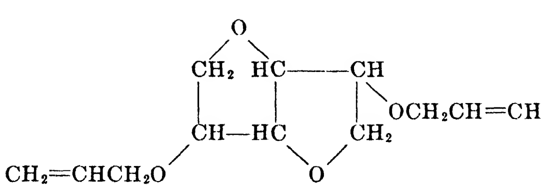


XVI

sorbitol is obtained by hydrogenation of sucrose or invert sugar, followed by dehydration of the mixture of mannitol and sorbitol in the presence of acid and an entraining liquid. The two dianhydrides are separated by fractional distillation. Inasmuch as the dianhydro-mannitol is produced



XVII



XVIII

(41) R. C. Hockett, Paper read at the Detroit meeting of the Am. Chem. Soc., April, 1943.

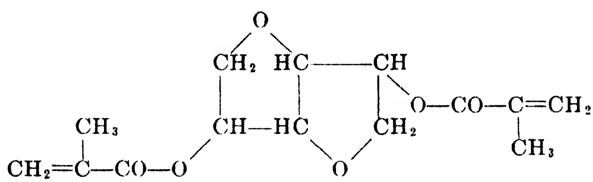
(42) H. G. Fletcher, Jr., and R. M. Goepp, Jr., *J. Am. Chem. Soc.*, **67**, 1042 (1945).

(43) R. Montgomery and L. F. Wiggins, *J. Chem. Soc.*, 390 (1946).

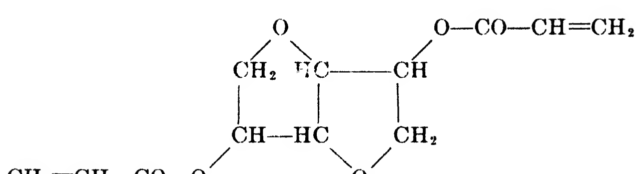
in only about 40% yield it may be advantageous to remove mannitol from the mixture of hexitols before dehydration, and to use it for other purposes. This can be done since mannitol crystallizes first and may be removed from a solution of the mixture in aqueous alcohol; the sorbitol residue may then be converted into dianhydro-sorbitol.

The methyl and ethyl ethers of the dianhydrides have properties which may make them useful as plasticizers.[44] 2,5-Diallyl-dianhydro-sorbitol (XVII) and 2,5-diallyl-dianhydro-mannitol (XVIII) and the corresponding methallyl derivatives polymerize to resinous materials on being heated in oxygen.[44] The allyl derivatives polymerize about five times as fast as the methallyl derivatives, to give products somewhat similar to that obtained by Nichols and Yanovsky[45] by the polymerization of methyl tetraallyl-α-D-glucoside.

Some interesting resins have been obtained from the dianhydrides through their acrylic or methacrylic esters.[46] Thus, 2,5-dimethacrylyl-1,4:3,6-dianhydro-mannitol (XIX) was formed by treating dianhydro-mannitol with methacrylyl chloride in the presence of sodium hydroxide. The product, a crystalline compound, polymerized extremely rapidly to a colorless, transparent, infusible resin. The corresponding sorbitol derivative, however, was an oil which polymerized to a softer resin. On the other hand, 2,5-diacrylyl-1,4:3,6-dianhydro-sorbitol (XX) was a crystalline compound, which polymerized to a hard, infusible, glass-like resin.



XIX



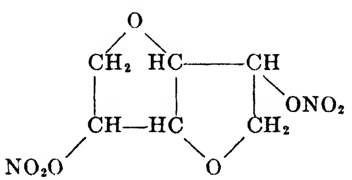
XX

The real importance of these compounds probably lies, however, in the fact that they copolymerize with methyl methacrylate and give a product

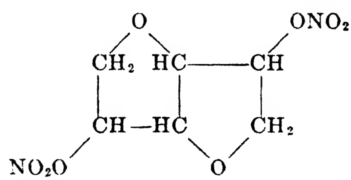
(44) W. N. Haworth and L. F. Wiggins, Brit. Pat. 599,048 (1945).
(45) P. L. Nichols and E. Yanovsky, *J. Am. Chem. Soc.*, **66**, 1625 (1944).
(46) W. N. Haworth, Hilda Gregory and L. F. Wiggins, *J. Chem. Soc.*, 488 (1946).

which is harder than pure methyl methacrylate polymer. The dianhydrides form crystalline esters with long-chain fatty acids such as palmitic and stearic acid and these esters are surface-active agents of some importance. Such substances as these and also esters of the anhydrides of hexahydric alcohols with unsaturated long-chain acids are industrially important as is evidenced by the commercial literature on the subject.

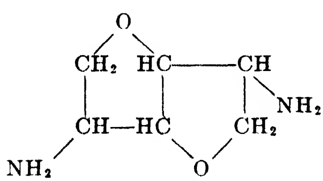
The dianhydrides of mannitol and sorbitol also form two crystalline dinitrates[47] (XXI) and (XXII), both of which are blood-pressure depressants. Curiously enough the sorbitol derivative is twice as active as the mannitol derivative, although both are less active than glycerol trinitrate.[48] Both anhydrides give rise to amino derivatives,[49] 2,5-



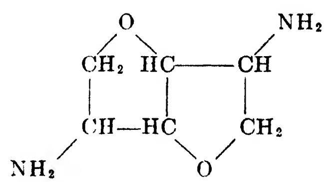
XXI



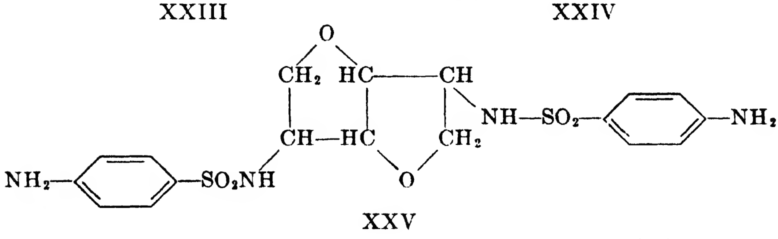
XXII



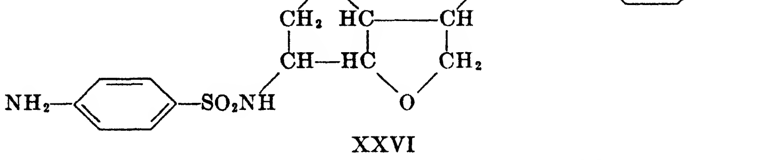
XXIII



XXIV



XXV



XXVI

(47) S. E. Forman, C. J. Carr and J. C. Krantz, Jr., *J. Am. Pharm. Assoc.*, **30**, 132 (1941).

(48) R. Montgomery and L. F. Wiggins, unpublished work.

(49) R. Montgomery and L. F. Wiggins, *J. Chem. Soc.*, 393 (1946).

diamino-1,4:3,6-dianhydro-mannitol (XXIII) and 2,5-diamino-1,4:3,6-dianhydro-sorbitol (XXIV), which have been converted into the corresponding sulfanilamides, (XXV) and (XXVI), respectively. These, however, were not particularly active in bacteriostatic tests.

3. *Acid Transformation Products*

It was found as long ago as 1840[50] that sucrose, when heated with mineral acids at high temperature gave a substance first called glucinic acid and later known as levulinic acid (XXVII).

$$CH_3—CO—CH_2—CH_2—COOH$$

XXVII

Levulinic acid is produced by the action of acids on most carbohydrates; thus glucose,[51] galactose,[52] sucrose,[53] fructose,[54] glucosamine,[55] chitose,[56] sorbose,[56] desoxypentoses[57] and hexose sugars as such or joined in disaccharide or polysaccharide union,[58] all give rise to it. Tollens and Conrad and their coworkers published many papers dealing with the formation of this substance from carbohydrates during the period 1874–1900. High yields of levulinic acid were not reported, the authors being primarily concerned with the types of carbohydrates which produced it. Variations of the method of preparation of levulinic acid have appeared in more recent times. Thus, McKenzie[59] obtained it in 22% yield by heating sucrose with concentrated hydrochloric acid at atmospheric pressure. Thompson[60] found that addition of sodium chloride to the hydrochloric acid solution of sucrose or other carbohydrate somewhat

(50) G. J. Mulder, *J. Prakt. Chem.*, **21,** 219 (1840).

(51) A. von Grote and B. Tollens, *Ann.*, **206,** 229 (1881); M. Conrad and M. Guthzeit, *Ber.*, **19,** 2569 (1886); E. Erlenmeyer, *J. Prakt. Chem.*, **71,** 382 (1905).

(52) W. H. Kent and B. Tollens, *Ann.*, **227,** 228 (1885); M. Conrad and M. Guthzeit, *Ber.*, **18,** 2906 (1885).

(53) A. von Grote and B. Tollens, *Ann.*, **175,** 183 (1875); A. von Grote, E. A. Kehrer and B. Tollens, *Ann.*, **206,** 209 (1881); M. Conrad and M. Guthzeit, *Ber.*, **18,** 439 (1885); M. Conrad, *Ber.*, **11,** 2178 (1878).

(54) A. von Grote and B. Tollens, *Ann.*, **175,** 195 (1875); M. Conrad and M. Guthzeit (ref. 51); E. Erlenmeyer (ref. 51).

(55) H. Hamburger, *Biochem. Z.*, **36,** 1 (1911).

(56) C. Wehmer and B. Tollens, *Ann.*, **243,** 320 (1887); R. H. Smith and B. Tollens, *Ber.*, **33,** 1285 (1900).

(57) P. A. Levene and T. Mori, *J. Biol. Chem.*, **83,** 803 (1929).

(58) C. Wehmer and B. Tollens (ref. 56); A. von Grote and B. Tollens (ref. 53); F. Bente, *Ber.*, **8,** 416 (1875); P. Rischbeit and B. Tollens, *Ann.*, **232,** 193 (1886); M. Lüdtke, *Biochem. Z.*, **212,** 419 (1929).

(59) B. F. McKenzie, *Organic Syntheses*, **9,** 50 (1929).

(60) A. Thompson (to Corn Products Refining Co.), Brit. Pat. 529,262 (1940).

increased the yield. Thomas and Schuette[61] showed that, if sucrose was digested under pressure with dilute hydrochloric acid at 162° for one hour, levulinic acid was obtained in 42% yield. The optimal concentration of hydrochloric acid was said to be 4.6%. The concentration of sucrose used was high; in this particular case it was 250 g. of sucrose in 600 cc. of acid solution. The writer[62] has also studied the reaction between sucrose and mineral acids and has shown, by carrying out experiments under comparable conditions (see Table II), that the efficacy of the different acids is as follows: HBr > HCl > H_2SO_4.

TABLE II

Action of Various Acids on Sucrose under the Same Experimental Conditions

Acid	*Concentration of sucrose, w/v per cent*	*Concentration of acid, w/v per cent*	*Yield of levulinic acid (per cent)*
H_2SO_4	6	9	42 3
HCl	6	9 7	60 0
HBr	6	9	70 0

(Sucrose is heated in sealed tubes with the acid solution at 125° for sixteen hours)

It has been shown also that the concentration of sucrose is a critical factor and that to obtain the highest yields very dilute solutions are necessary. Thus Fig. 1 shows the relative yields at different concentrations of sucrose heated with hydrobromic acid, all experiments being carried out under similar conditions. The highest yield of levulinic acid, weighed as crude material, was 79% of the theoretical and was obtained when the concentration of sucrose was 3%. Ploetz[63] has also used the hydrobromic acid method to make levulinic acid and records a yield of 69% of the theoretical from crude sugar, 75% from glucose and 64% from starch.

Hydrobromic acid, although it shows marked advantages over other acids in the preparation of levulinic acid, is obviously too expensive for the manufacture of this material. Some improvement in the yield obtained with hydrochloric acid is effected, however, by adding a small amount of sodium bromide to the mixture.[64]

The mechanism of the formation of levulinic acid probably involves the substance 5-hydroxymethylfurfural (XXVIII), which may also be obtained from sucrose by treatment with dilute oxalic acid.[65] This

(61) R. W. Thomas and H. A. Schuette, *J. Am. Chem. Soc.*, **53**, 2324 (1931).
(62) L. F. Wiggins, in press.
(63) T. Ploetz, German Pat. 732,896 (1943).
(64) W. N. Haworth and L. F. Wiggins, Brit. Pat. 583,533 (1944).
(65) J. Kiermayer, *Chem. Ztg.*, **19**, 1003 (1895).

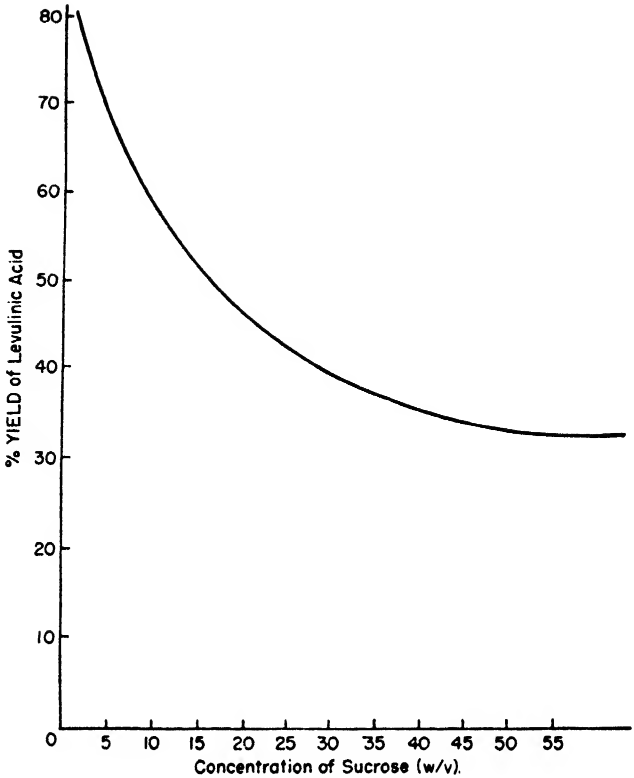
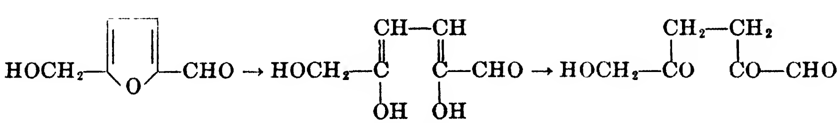


FIG. 1.

substance is known to suffer degradation to levulinic acid in the presence of mineral acids or even of concentrated oxalic acid,[66] probably via the intermediates XXIX → XXXI.



XXVIII XXIX XXX

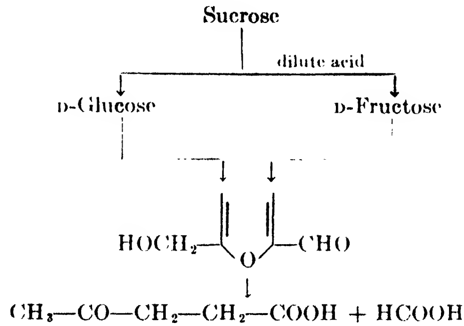
$$CH_3—CO—CH_2—CH_2—COOH \leftarrow HOCH_2—CO—CH_2—CH_2—CHO + HCOOH$$

XXXI

Hydroxymethylfurfural is produced by the action of oxalic acid, at a high temperature and under pressure, on either fructose or glucose, although it is more readily obtained from the ketose. No hydroxy-

(66) H. P. Teunissen, *Rec. trav. chim.*, **49**, 784 (1930).

methylfurfural is isolated when carbohydrates are treated with mineral acid under these conditions. The course of the reaction can then be represented diagrammatically as shown below.



It is clear that for every mole of levulinic acid produced there is an equal amount of formic acid; thus if appropriate methods of isolation are employed both of these products may be obtained from sucrose. The isolation of levulinic acid can be accomplished either by partial neutralization, filtration of humin material and vacuum steam distillation according to the Moyer process,[67] or by solvent extraction. Macallum[68] suggests methylene chloride and also butanol as a suitable solvent for this purpose. The levulinic acid obtained by the second method is usually a dark brown crystalline solid which is subsequently distilled. This product and that obtained by the first method are light yellow-colored liquids and recently Moyer[69] has described a method of decolorizing levulinic acid by treating it with a very small amount of oxidizing agent, sodium chlorite ($NaClO_2$) and hydrogen peroxide. In the writer's experiments solvent extraction was employed to isolate both levulinic acid and formic acid. Yields of formic acid of 60 to 70% of the theoretical were quite easily obtained, and having regard to the possible uses of the esters of formic acid this material is a by-product of some value. Another process for levulinic acid preparation is due to Weidenhagen, Korotkyi and Spengler,[70] who heated sucrose at 160 to 165° in methanol containing 2.5% of hydrogen chloride and sodium sulfate and obtained methyl levulinate in 21% yield.

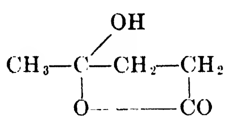
Levulinic acid therefore can be obtained by several processes from sucrose and D-glucose in fairly high yield. Moreover the operations necessary to effect this transformation offer no unsurmountable diffi-

(67) W. W. Moyer (to Staley Manufacturing Co.), U. S. Pat. 2,270,328 (1942).
(68) A. D. Macallum (to E. I. du Pont de Nemours), U. S. Pat. 2,257,389 (1941).
(69) W. W. Moyer (to Staley Manufacturing Co.), U. S. Pat. 2,349,514 (1944).
(70) R. Weidenhagen, B. Korotkyi and O. Spengler, German Pat. 635,783 (1936).

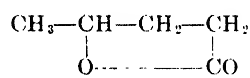
culties to the chemical engineer. Indeed, one American firm is in a position to manufacture levulinic acid on a large scale, were the demand for it to arise. Levulinic acid lends itself to innumerable chemical conversions and provides access, as will be shown later, to materials some of which have, and others of which may have, marked usefulness.

There is one use to which levulinic acid can immediately be put. We have found that its sodium salt has ideal properties for an antifreeze agent. It has definite advantages over ethylene glycol for this purpose. It is a solid and is therefore more easily marketed than the liquid glycol. It is less corrosive to the iron parts of internal combustion engines than is tap water itself and has no detrimental effect on the rubber connections used in engines.

Levulinic acid is highly reactive and moreover is capable of assuming a lactone form (XXXII). Valeric γ-lactone (XXXIII) can be obtained in very high yield by hydrogenation of levulinic acid,[71,72] and this compound is a good solvent and may well find extensive uses as such. Moreover it may be hydrogenated to 1,4-pentandiol (XXXIV),[73] which on dehydration yields 1,3-pentadiene (piperylene) (XXXV). Piperylene is



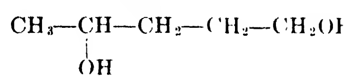
XXXII



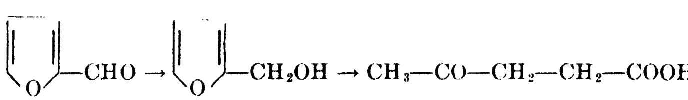
XXXIII

$CH_3—CH=CH—CH=CH_2$

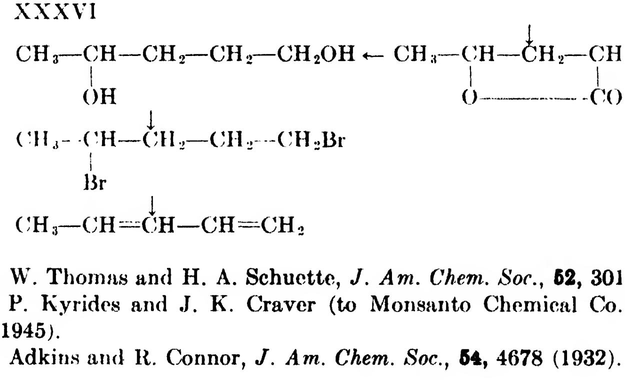
XXXV



XXXIV



XXXVI



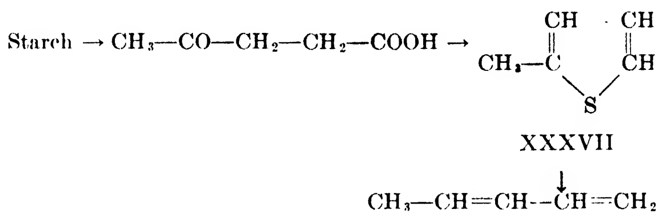
(71) R. W. Thomas and H. A. Schuette, *J. Am. Chem. Soc.*, **52**, 3010 (1930).

(72) L. P. Kyrides and J. K. Craver (to Monsanto Chemical Co.), U. S. Pat. 2,368,366 (1945).

(73) H. Adkins and R. Connor, *J. Am. Chem. Soc.*, **54**, 4678 (1932).

known to polymerize to a rubbery mass. Berkenheim and Dankova[74] obtained it from furfural (XXXVI) as shown below, and claimed it as a source of synthetic rubber.

It is interesting to note that a similar scheme for making synthetic rubber was put forward many years ago. This Heinemann synthetic rubber process was essentially the production of levulinic acid from starch, its conversion with phosphorus pentasulfide into methylthiophene (XXXVII), which is reduced to 1,3-pentadiene.



XXXVII

Many esters of levulinic acid are assuming industrial importance. Cox and Dodds[75] in 1933 described such esters as the butyl, hexyl etc. The cyclohexyl ester is mentioned in the "Plastics Catalogue" and has recently been advertised by Monsanto Chemical Co. as a plasticizer. Other long-chain alkyl esters[76] have been described as plasticizers for cellulose esters. Cellulose ester plasticizers[77] have also been made by esterifying polyhydric alcohols containing one free hydroxyl group, with levulinic acid. The methyl, isopropyl, isoamyl and 2-pentanol esters of levulinic acid were described by Stampa[78] as solvents for synthetic glass-like resins. The use of levulinic acid esters as constituents of hydraulic-brake fluids and as solvents for the extraction of glyceride oils, such as linseed, soya bean and fish oils, has been suggested.[79]

Several uses have been suggested for levulinic acid and its salts. Thus, calcium levulinate seems to have advantages as a calcium carrier in tuberculosis therapy, and it is said to be more suitable than calcium gluconate for intravenous injection.[80] A mercury salt, phenyl mercury

(74) A. M. Berkenheim and T. F. Dankova, *J. Gen. Chem., U.S.S.R.*, **9**, 924 (1939); *Chem. Abstracts*, **34**, 368 (1940).

(75) G. J. Cox and M. L. Dodds, *J. Am. Chem. Soc.*, **55**, 3391 (1933); *Ind. Eng. Chem.*, **25**, 967 (1933).

(76) W. E. Lawson and P. L. Salzberg (to E. I. du Pont de Nemours), U. S. Pat. 2,008,720 (1935).

(77) E. F. Izard and P. L. Salzberg (to E. I. du Pont de Nemours), U. S. Pat. 2,004,115 (1935).

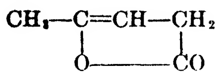
(78) G. Stampa, *Intern. Sugar J.*, **41**, 270 (1939).

(79) R. R. Fulton (to Puritan Soaps Co.), U. S. Pat. 1,986,260 (1935).

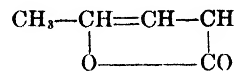
(80) A. Proskouriakoff, *J. Am. Chem. Soc.*, **55**, 2132 (1933).

levulinate, can be used as a detergent and in germicidal soaps.[81] Levulinic acid itself has been described as a solvent for the aromatic constituents of crude mineral oil.[82] The combination of levulinic acid with organic amines provides derivatives of some importance. Meigs has been granted a patent[83] for the use of the addition product of levulinic acid and ethanolamine as a softener for certain cellulose materials. By condensing levulinic acid with an organic amine a useful synthetic resin results.[84]

Levulinic acid, acting in its lactone form (XXXII) undergoes dehydration and two products have been obtained, α- and β-angelica lactones, (XXXVIII) and (XXXIX), respectively. α-Angelica lactone has been found to polymerize[85] to a tacky resin by the catalytic agency of boron trifluoride.



XXXVIII



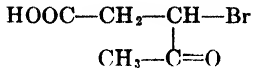
XXXIX

Levulinic acid is fairly easily converted into thiazole derivatives by the intermediate formation of an α-halogenated ketone such as the β-bromo derivative (XL) or β-chloro derivative, which reacts with thiourea to form 2-amino-4-methyl-5-thiazoleacetic acid (XLI) or with thioformamide to give 4-methyl-5-thiazoleacetic acid (XLII).[86] The aminothiazole (XLI) and its ethyl ester (XLIII) have been converted into their corresponding sulfanilamide derivatives,[87] (XLIV) and (XLV). These sulfanilamides, particularly the acid XLIV, have considerable chemotherapeutic activity; moreover the acid possesses distinct solubility advantages over sulfathiazole itself.

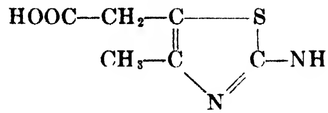
Another series of compounds which are easily accessible from levulinic acid are pyridazone and pyridazine derivatives. Levulinic acid and its esters condense almost quantitatively with hydrazine to give 6-methyl-3-pyridazinone (XLVI), which is readily dehydrogenated by means of bromine to give 6-methyl-3-pyridazone (XLVII).

Chlorination of the pyridazone yields 3-chloro-6-methyl-pyridazine (XLVIII) which on treatment with ammonia gives 3-amino-6-methyl-pyridazine (XLIX). The sulfanilamide derivative (L) has promising

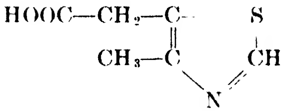
(81) Lever Bros. Ltd., Brit. Pat. 427,324 (1935).
(82) F. X. Govers, U. S. Pat. 2,087,473 (1937).
(83) F. M. Meigs (to E. I. du Pont de Nemours), U. S. Pat. 2,191,897 (1940).
(84) A. G. Hovey and T. S. Hodgins, U. S. Pat. 2,195,570 (1940).
(85) C. S. Marvel and C. L. Levesque, *J. Am. Chem. Soc.*, **61**, 1682 (1939).
(86) L. R. Cerecedo and J. G. Tolpin, *J. Am. Chem. Soc.*, **59**, 1660 (1937).
(87) Hilda Gregory and L. F. Wiggins, *J. Chem. Soc.*, 590 (1947).



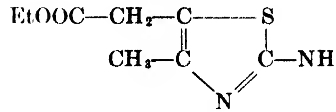
XL



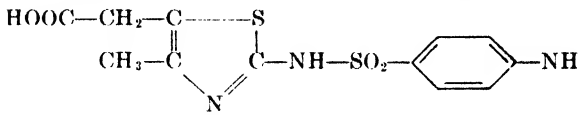
XLI



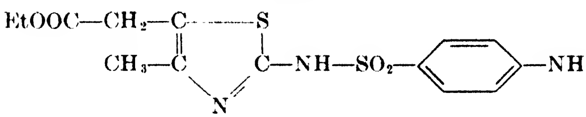
XLII



XLIII



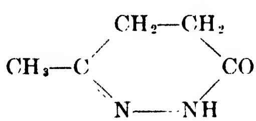
XLIV



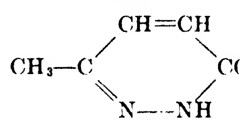
XLV

properties as an antibacterial agent.[87a,88] Moreover it possesses desirable pharmacological properties in so far as both it and its *N*-acetyl derivative are many times more soluble than sulfathiazole.

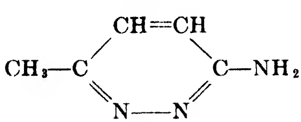
Other derivatives of this class of compound show marked analgesic and antipyretic activity. Thus 2,6-dimethyl-3-pyridazone (LI, R = CH_3)



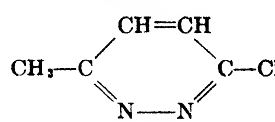
XLVI



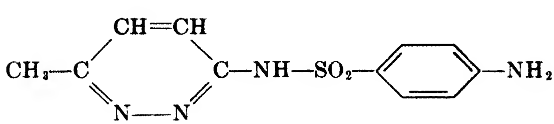
XLVII



XLIX



XLVIII

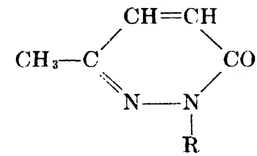


L

(87a) W. G. Overend and L. F. Wiggins, *J. Chem. Soc.*, 239 (1947).

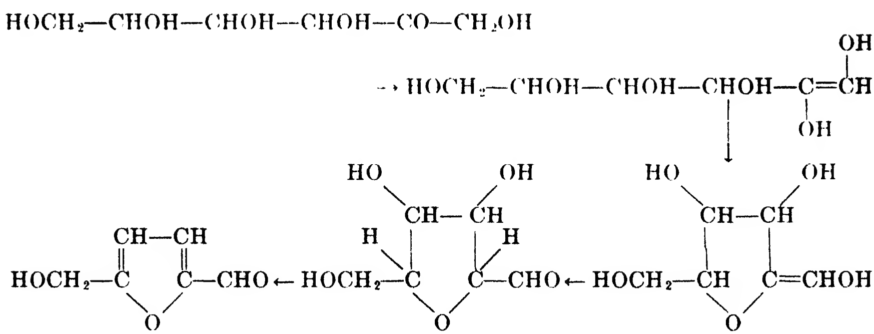
(88) R. O. Roblin and P. S. Winnek (to American Cyanamide Co.), Brit. Pat. 563,629 (1944).

and 3-chloro-6-methyl-pyridazine (XLVIII) are of about the activity of phenazone as analgesics, and 2-butyl-6-methyl-3-pyridazone (LI, R = C_4H_9) is a particularly powerful analgesic. The 2-alkyl derivatives also show marked local anaesthetic properties, the 2-amyl (LI, R = C_5H_{11}) and the 2-(diethylaminoethyl) derivatives (LI, R = Et_2N-$(CH_2)_2$) being of particular interest.[89]



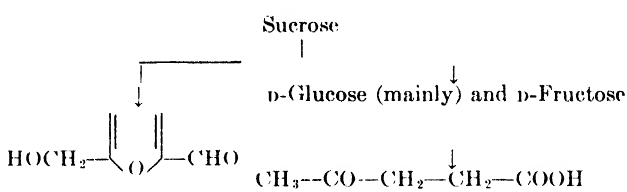
LI

5-Hydroxymethylfurfural (XXVIII), which may be regarded as an acid degradation product of sucrose, was first prepared by Dull[90] in 1895 by heating fructose with dilute oxalic acid. Kiermayer[91] in the same year obtained it from sucrose by similar treatment. It was later obtained from several other carbohydrates such as sorbose,[92] chitose[93] and even cellulose.[94] It is obtained more readily from ketoses than from aldoses, although even with ketoses high yields of hydroxymethyl-furfural are not obtained. In a paper published in 1944, Haworth and Jones[95] suggested a mechanism for the production of hydroxymethylfurfural from sucrose involving the 1,2-enediol of glucose or fructose as an essential feature. The successive steps in the conversion are shown below:



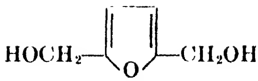
(89) W. N. Haworth and L. F. Wiggins, Patent applied for.
(90) G. Dull, *Chem. Ztg.*, **19**, 216 (1895).
(91) J. Kiermayer, *Chem. Ztg.*, **19**, 1003 (1895).
(92) E. Votoček, *Ber.*, **30**, 1195 (1897).
(93) W. Alberda van Ekenstein and J. J. Blanksma, *Ber.*, **43**, 2355 (1910).
(94) E. Heuser, *Cellulosechemie*, **4**, 15, 101 (1923).
(95) W. N. Haworth and W. G. M. Jones, *J. Chem. Soc.*, 667 (1944).

More recently Montgomery and Wiggins[96] have studied the effect of acids other than oxalic on sucrose and have found that not only acids such as maleic, fumaric and phosphoric (which have dissociation constants of the same order as that of oxalic acid) but also hydrochloric acid in dilution such that the pH of the solution was the same as that of the other acids mentioned above, produced 5-hydroxymethylfurfural. Furthermore, when sucrose was heated in water at a temperature of 130 to 170° sufficient acidity developed to cause inversion and then degradation to 5-hydroxymethylfurfural. The yield of 5-hydroxymethylfurfural was about the same for all the acids used. It is interesting to note that an atmosphere of hydrogen aids the reaction, whereas one of nitrogen does not. One hundred grams of sucrose under the best conditions yields 20 g. of 5-hydroxymethylfurfural together with a red humin material, the amount of which can be kept as low as 0.5 to 1 g. The remaining sucrose is present as a mixture of fructose and glucose with glucose predominating. It would seem indeed that under these conditions none of the glucose component is converted into hydroxymethylfurfural. These residual sugars of course can be utilized for other purposes, for instance after extraction of the 5-hydroxymethylfurfural, they can be treated with hydrochloric acid containing sodium bromide and converted to levulinic acid. 5-Hydroxymethylfurfural, therefore, can be considered as a potential intermediate in the utilization of sucrose since we have the following process starting with sucrose:

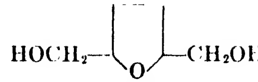


5-Hydroxymethylfurfural is a labile substance, very different from furfural itself, although in the early literature there appears to be some confusion between the two. Its most distinctive property is the ease with which it undergoes ring scission, but numerous other reactions are possible. Perhaps the most important of these is that of hydrogenation. By hydrogenation at fairly low temperature with Raney nickel, it can be made to yield furan 2,5-dicarbinol (LII), which also is a rather unstable substance, in this respect resembling furfuryl alcohol. However, if hydrogenation is carried out at higher temperatures with the same catalyst, saturation of the furan nucleus occurs with the formation of

(96) R. Montgomery and L. F. Wiggins, *J. Soc. Chem. Ind. London*, **66**, 31 (1947).

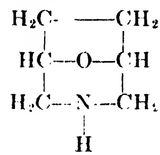


LII



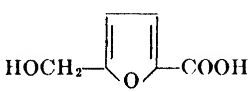
LIII

tetrahydrofuran 2,5-dicarbinol (LIII).[97] This compound is of some importance since it is a stable, colorless liquid of high boiling point, which may find uses as a plasticizer for certain synthetic resins. On attempting to prepare a diamine from this substance an unusual product was obtained. When a crystalline ditosyl derivative of the compound LIII was treated with ammonia it gave, instead of the expected diamine, a bicyclic compound represented by LIV, which might be considered as a derivative of hexamethyleneimine.[98]

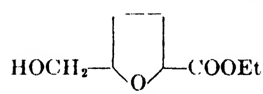


LIV

5-Hydroxymethylfurfural may be oxidized to 5-hydroxymethylfuran-2-carboxylic acid (LV), the ethyl ester of which is easily hydrogenated to ethyl 5-hydroxymethyltetrahydrofuran-2-carboxylate (LVI). This ester is a potential source of adipic acid by the following series of

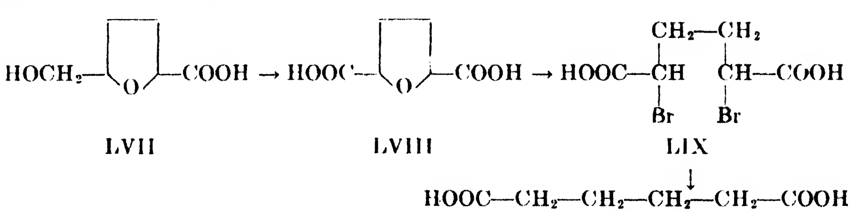


LV



LVI

reactions.[97] The ester (LVI) is hydrolyzed to the acid (LVII) which is then oxidized with nitric acid to tetrahydrofuran-2,5-dicarboxylic acid (LVIII). This dibasic acid undergoes ring scission with hydrogen bromide in acetic acid to give 2,5-dibromoadipic acid (LIX), hydrogenation of which gives adipic acid (LX).



LVII LVIII LIX LX

(97) W. N. Haworth, W. G. M. Jones and L. F. Wiggins, *J. Chem. Soc.*, 1 (1945).
(98) F. H. Newth and L. F. Wiggins, *J. Chem. Soc.*, 155 (1948).

4. *Alkali Degradation of Sucrose*

The action of alkaline reagents on sugars has been the subject of much study since Lobry de Bruyn's researches in 1896.[99] Many complex changes are induced in the sugar molecule by alkaline reagents, as is exemplified by the researches of Evans.[100] Here, however, mention will be made only of the production of lactic acid by the action of alkali on sucrose.

That lactic acid is produced by the action of alkali on sugars has been known for many years. Nef, a pioneer in this study, often obtained it in his experiments. Later Kolbach, Ruckdeschel and Windisch[101] obtained lactic acid in 48% yield from sucrose by treating it with 0.5 *N* sodium hydroxide solution. More extensive work was carried out by Wolf,[102] who treated sucrose with lime water at high temperatures and showed, by estimation, that lactic acid was produced in 73% of the theoretical yield, although only 60% was actually isolated as the zinc salt. One firm[103] has patented a process for the production of lactic acid by a method in which sucrose is heated with two molecular proportions of lime at 200 to 250° and 20 to 30 atmospheres pressure for 1.5 hours. It is claimed that the reaction mixture contains 72 to 75% of the theoretical amount of lactic acid but the yield of zinc lactate actually isolated appears to be only 50%. Haworth, Gregory and Wiggins[104] have investigated the reaction between cane sugar and lime with a view to finding the optimum conditions for lactic acid formation. A number of experiments were carried out in which temperature, time of heating, the amount of lime and the concentration of sucrose were varied. It was found that the maximum yield of lactic acid was obtained on heating for seven hours a 40% solution of sucrose with 2.25 moles of lime at 225° to 240° and a pressure of 30 atmospheres. Under these conditions it was possible to isolate zinc lactate (twice recrystallized) in 71% of the theoretical yield. There is thus made available a chemical process for the manufacture of lactic acid which might well compete with the established fermentation method (see page 329). By whatever means it is prepared, lactic acid is an important intermediate in the utilization of

(99) C. A. Lobry de Bruyn and W. Alberda van Ekenstein, *Rec. trav. chim.*, **15**, 93 (1896).

(100) W. L. Evans, *Chem. Revs.*, **31**, 537 (1942).

(101) P. Kolbach, H. Ruckdeschel and W. Windisch, *Wochschr. Brau.*, **44**, 405 (1927).

(102) H. Wolf, *Biochem. Z.*, **210**, 458 (1929); **219**, 232 (1930).

(103) Standard Brands Inc., Brit. Pat. 400,413 (1933).

(104) W. N. Haworth, Hilda Gregory and L. F. Wiggins, *J. Soc. Chem. Ind. London*, **65**, 95 (1946).

surplus sucrose. Many uses of lactic acid are already known and these could be extended should the availability of lactic acid increase. At present the largest user of lactic acid is the leather industry, but the esters of lactic acid are also used as plasticizers and solvents; for example Morell[105] describes glycerol lactate as a plasticizer for cellulose derivatives and Graves proposes a similar use for dodecyl lactate in a patent issued in 1938.[106] Moreover, sucrose can be converted through lactic acid into synthetic resins. Burns, Jones and Ritchie[107] in 1935 showed that esters of acetylated lactic acid are convertible in high yield into esters of acrylic acid by pyrolysis. More recently Fisher and coworkers[108] have studied the conversion of lactic acid (LXI) through methyl acetoxypropionate (LXII) into methyl acrylate (LXIII), which polymerizes to give soft

COOH	COOMe	COOMe
|	|	|
HCOH	HCOAc	HC
|	|	‖
CH_3	CH_3	CH_2
LXI	LXII	LXIII

resins distinct from the hard glass-like resins obtained from methyl methacrylate. Fisher[109] and his coworkers have examined the co-polymerization of methyl acrylate with substances such as butadiene, isoprene and allyl maleate to form rubber-like products which can be vulcanized to give excellent rubber substitutes. To these, the name Lactoprene has been given to emphasize the part played by lactic acid in their formation. An alternative route for the conversion of cane sugar into synthetic rubber is through acrylonitrile, a well-known component of synthetic rubbers, which can be obtained from acetoxypropiononitrile, itself prepared from lactic acid.

Besides the acrylate resins, lactic acid can be converted into other resinous products, which have been covered in the brochure by Long;[34] it is clear that synthetic resins, rubber and plasticizers are obtainable from lactic acid and thus ultimately from sucrose.

IV. Derivatives of Sucrose

The most important of the acyl derivatives of sucrose is doubtless sucrose octaacetate. This is represented by formula LXIV, the consti-

(105) R. S. Morell, "Synthetic Resins and Allied Plastics," Oxford Univ. Press, p. 300 (1943).

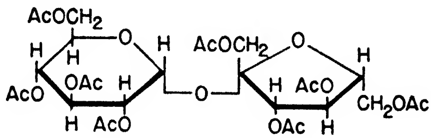
(106) G. D. Graves (to E. I. du Pont de Nemours), U. S. Pat. 2,122,716 (1938).

(107) R. Burns, D. T. Jones and P. D. Ritchie, *J. Chem. Soc.*, 400 (1935).

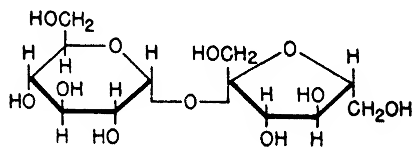
(108) C. H. Fisher, W. P. Ratchford, L. T. Smith and M. L. Fein, *Ind. Eng. Chem.*, **34**, 473 (1942); C. H. Fisher, Chessie E. Rehberg and L. T. Smith, *J. Am. Chem. Soc.*, **65**, 763 (1943).

(109) C. H. Fisher, W. G. Mast, Chessie E. Rehberg and L. T. Smith, *Ind. Eng. Chem.*, **36**, 1032 (1944).

tution of sucrose as determined by Haworth and his coworkers[110,111] being given by LXV. The octaacetate is a crystalline substance of melting point 85°, which on melting and cooling yields a colorless hard resin. It is this property of sucrose octaacetate that makes it important. It can be used in making laminated glass or incorporated into such synthetic resins as cellulose acetate, polymeric methyl methacrylate and



LXIV



LXV

polyvinyl acetate, etc. Among other uses for sucrose octaacetate, one might mention that mixtures of sucrose octaacetate and the acetates of glucose and fructose produced by the acetylation of partly inverted sucrose may be used as adhesives.[112] Sucrose octaacetate has an extremely bitter taste, and this property has suggested its use as a substitute for bitters and as denaturant for alcohol, as it has been shown to be quite innocuous to man.[113] The acetylation of sucrose has occupied a number of investigators. Thus, Cox, Dodds and Ferguson[114] studied the action of acetic anhydride and sodium acetate on sucrose and described large-scale experiments. Seymour[115] patented such a process as a general one for the acetylation of sugars and gives as an example sucrose octaacetate. British Celanese Ltd.[116] has also obtained a patent based on essentially the same facts and claim a yield of 80% of the theoretical. On the other hand Sandera[117] has used pyridine as a catalyst and Amagasa and Yanagita[118] claim that this gives better yields than the sodium acetate–acetic

(110) W. N. Haworth and J. Law, *J. Chem. Soc.*, **109**, 1314 (1916).
(111) J. Avery, W. N. Haworth and E. L. Hirst, *J. Chem. Soc.*, 2308 (1927).
(112) V. Walthausen and Lylla R. Metry, U. S. Pat. 2,174,541 (1939).
(113) M. W. Green, *Bull. Natl. Formulary Comm.*, **10**, 131 (1942), **11**, 59 (1943).
(114) G. J. Cox, M. L. Dodds and J. H. Ferguson, *Ind. Eng. Chem.*, **25**, 968 (1933).
(115) G. W. Seymour (to Camille Dreyfus), Can. Pat. 428,307 (1945).
(116) British Celanese Ltd., Brit. Pat. 552,161 (1943).
(117) K. Sandera, *Chem. Listy*, **33**, 139 (1939).
(118) M. Amagasa and T. Yanagita, *J. Soc. Chem. Ind. Japan*, **43**, 444 (1940).

anhydride method. Leman[119] states that acetylation is quantitative in the presence of pyridine.

Sucrose octapropionate and octabutyrate have also been prepared but have not been examined so extensively as the acetate. Hurd and Gordon[120] obtained the octapropionate in crystalline form, whereas the octabutyrate was obtained by Cox and coworkers[114] and more recently by Wolff[121] as a liquid. Both of these products, in view of their very high boiling points, may be useful as plasticizing agents. The ester with methacrylic acid would be of interest but so far attempts made in these laboratories to obtain a monomeric substance have failed and only an insoluble polymer is obtained.

Among the several other esters of sucrose that have been described, the octanitrate deserves some mention. This substance has been known for many years and has possible uses as an explosive, although Marshall[122] states that it is probably too unstable in its sirupy form to be used as an explosive. Since that time, however, new methods of manufacture have been described and the octanitrate of sucrose may be obtained in a highly purified and crystalline form. The nitration is carried out with a nitric acid–sulfuric acid mixture in the presence of nitrated butyl lactate and carbon tetrachloride.[123] By using different proportions of these solvents and also others, the form in which sucrose octanitrate separates can be varied at will so that it is possible to obtain the nitrated sugar in a form suitable for blasting operations.

Of the alkyl derivatives of sucrose, the heptamethyl and octamethyl derivatives were obtained by Haworth and coworkers during the early constitutional work on sucrose. It should be possible to produce these ethers on a larger scale, and since they are high-boiling liquids they might be useful plasticizers. Benzyl ethers of sucrose were obtained by Gomberg and Buchler[124] by treating sucrose with benzyl chloride and sodium hydroxide; the products were mainly dibenzylsucrose and pentabenzylsucrose, and these compounds, like the octaacetate, possess an intensely bitter taste. More recently the allylation of sucrose has been attempted by Yanovsky and Nichols[125] who obtained heptaallyl sucrose and purified it by molecular distillation. This compound undergoes

(119) A. Leman, *Compt. rend.*, **214,** 84 (1942).
(120) C. D. Hurd and K. M. Gordon, *J. Am. Chem. Soc.*, **63,** 2656 (1941).
(121) I. A. Wolff, *J. Am. Chem. Soc.*, **67,** 1623 (1945).
(122) A. Marshall, "Explosives," J. and A. Churchill, London, Vol. I, p. 197 (1917).
(123) J. A. Wyler (to Trojan Powder Co.), U. S. Pat. 2,101,927 (1937).
(124) M. Gomberg and C. C. Buchler, *J. Am. Chem. Soc.*, **43,** 1904 (1921).
(125) E. Yanovsky and P. L. Nichols, *J. Am. Chem. Soc.*, **67,** 47 (1945).

polymerization in the presence of gaseous oxygen, and may be of some importance in coating compositions.

V. Utilization of Sucrose Itself

Attempts to use sucrose itself for other purposes than as a food or sweetening agent cannot be said to have met with much success in the past.

Powell[126] in 1904 found that if wood was treated with sucrose solution and subsequently dried at a high temperature, it did not shrink. By this process Powell claimed to strengthen the fibers of the wood. Rice[127] believes that the treatment reduces the cell spaces and stabilizes the wood, thereby minimizing shrinkage and the possibility of rot. An example of the process is as follows:

The wood is immersed in a solution made by dissolving 360 lb. of semi-invert sugar, 20 lb. of glucose and 15 lb. of sodium fluoride in 1200 lb. of water together with sodium carbonate equal to 0.5% of the weight of the solution. The immersed wood and the solution are heated to boiling and then allowed to cool; the wood is removed and dried. If it is required to be protected against all forms of mold and stain, then an additional 42 lb. of sodium fluoride is required.

Stamm[128] has made a thorough examination of the effect of sucrose and invert sugar on the relevant physical properties of wood and has found that shrinkage is definitely retarded by the deposition of the sugar in the wood structure. Invert sugar seems superior in this effect to sucrose itself.

Several workers have described both advantageous and detrimental effects obtained on adding sucrose to the mixing water for mortars. Tsountas[129] states that the addition of small amounts of sucrose to Portland cement mortars effects a decrease in the setting time but also lowers the strength of the resulting mortar and causes general weakening of the structure. Gonell,[130] too, finds that the addition of 0.1% of sucrose to Portland cement is definitely detrimental. On the other hand, Hall[131] made mortars consisting of hydrated lime (1 part), sand (4 parts) and water to which had been added 3% of cheap brown sugar. The mortar set very quickly and showed as good an adhesion to brick as Portland cement. Another investigation[132] was carried out at the

(126) W. S. Powell, U. S. Pat. 755,240 (1904).

(127) G. E. Rice, U. S. Pats. 1,527,330 (1922), 1,732,419 (1924), 1,732,420 (1924).

(128) A. J. Stamm, *Ind. Eng. Chem.*, **29**, 833 (1937).

(129) C. Tsountas, *Rec. mat. construction travaux publics*, **182**, 281 (1924); *Chem. Abstracts*, **19**, 2117 (1925).

(130) H. W. Gonell, *Zement*, **18**, 372, 472 (1929).

(131) L. G. Hall, *Eng. News-Record*, **108**, 222 (1932).

(132) W. A. Hamor, G. J. Cox and J. W. van Brunt, *J. Am. Ceram. Soc.*, **16**, 187 (1933).

Mellon Institute on the effect of adding sugar to sand-lime bricks. It was found that the addition of 6% sugar (this amounts to 13 lb. of sugar per 1000 bricks) increased the tensile strength by about 60%. To summarize, it seems that sucrose exerts a beneficial effect on mortar and on sand-lime brick but a detrimental effect on Portland cement.

Attempts to utilize sucrose in synthetic resins have had no great success. The position has recently been summarized by Long[34] in an article "Sugar and Sugar By-Products in the Plastics Industry." As far as the author is aware the only other possible use of sucrose as such is in solution as a lubricant for certain types of machinery used in the sugar industry, for example in rotary pumps and stirring apparatus.[133]

VI. Conversion of Sucrose into Other Products by Fermentation Processes

Since sucrose is an essential constituent of the nutrient medium for the culture of many microorganisms producing a wide variety of metabolic products, it is obvious that many of these products may have an important bearing on the problem of the utilization of sucrose. Many such products are already well known and their manufacture requires large amounts of carbohydrate materials, particularly of molasses. Ethyl alcohol, butanol and acetone are produced on a very large scale but there are other materials not so well known that can be made by the action of various microorganisms on sucrose-containing solutions and which are being developed at the moment. It is on these that particular emphasis

Table III

Compounds Produced in Relatively Large Amounts through the Action of Microorganisms on Sugars

Acidic compounds	*Monohydric and polyhydric alcohols*	*Ketones*
Acetic acid	Ethyl alcohol	Acetone
Propionic acid	Butyl alcohol	Acetylmethylcarbinol
Butyric acid	2,3-Butylene glycol	
Lactic acid	Glycerol	
Fumaric acid	Mannitol	
Itaconic acid		
Citric acid		
Kojic acid		
2-Ketogluconic acid		
5-Ketogluconic acid		
Gluconic acid		

(133) G. Hrudka, *Sugar News*, **13**, 207 (1932).

will be laid in this section. The table given above (Table III) indicates the variety of products that are obtainable with reasonable ease by the growth of various microorganisms on sucrose or glucose.

The ethyl alcohol fermentation is of course an age-old process and is so well known that little need be said about it here. The acetone–butanol fermentation is perhaps the next most important industrial fermentation process, although starch in the form of maize has been largely used as the basic material; more recently suitably treated molasses has been used. The fermentation, a relatively rapid process requiring about thirty hours, produces about 60 parts of butanol, 30 parts of acetone and 10 parts of ethyl alcohol. These products already have large uses in industry and other uses are being explored. One possibility is the use of butanol in motor fuel. Jean[134] has described a fuel, called Jeanite, consisting mainly of butanol and ethyl alcohol, which shows some promise. Of course the admixture of ethyl alcohol with petroleum is well known and an increased use of this mixture is probable.

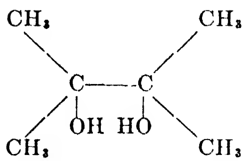
Butanol, which at one time was an unwanted by-product in the preparation of acetone, is now the most important product of the fermentation. The building of a large new factory in Puerto Rico using 10,000 tons of molasses per annum for its production is an indication of this importance. Butanol is probably still the best solvent for cellulose nitrate lacquers. Dibutyl phthalate is certainly the most widely used plasticizer for synthetic resins, and butyl oleate, tributyl citrate and dibutyl tartrate have also been described as plasticizers. Another important use of butanol is as a source of butadiene, which serves as an intermediate in the conversion of sucrose into a synthetic rubber. Although in recent years other methods have been described for the preparation of butanol (for example, from ethyl alcohol and from acetylene), yet the fermentation of carbohydrates is still the cheapest process.

Although acetone is used widely as an industrial solvent, nevertheless it has become the by-product of the acetone–butanol fermentation and there is always the fear of overproduction. There is thus a need for an extension of the industrial utilization of acetone. A possibility in this direction may be in its conversion into pinacol, the preparation of which has recently been improved by McHenry, Drum and O'Connor.[135] It is obtained together with isopropyl alcohol by electrolytic reduction of acetone under controlled conditions. Pinacol (LXVI) may be dehydrated to 2,3-dimethylbutadiene which can be converted into a synthetic rubber, or converted through pinacolone (LXVII) into neohexane

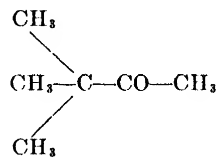
(134) J. W. Jean, U. S. Pat. 2,179,151 (1939).

(135) J. J. McHenry, P. J. Drum and W. F. O'Connor, *Proc. Roy. Irish Acad.*, **50B**, 219 (1945).

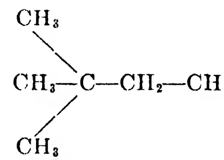
(LXVIII), a constituent of aviation fuel. The reaction of acetone with acetylene may also lead to developments of importance in finding new uses for acetone. For example, acetone reacts with acetylene in the presence of a metal acetylide catalyst to form the compound LXIX, which could be converted into isopentane (LXX), another constituent of aviation fuel. There may be possibilities of using the condensation products of acetone such as mesityl oxide (LXXI), which could be converted through the saturated ketone, methyl isobutyl ketone (LXXII), into 4-methylpentane (LXXIII).



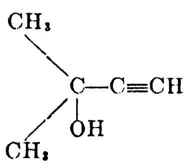
LXVI



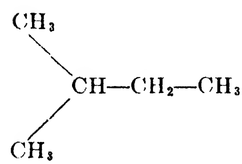
LXVII



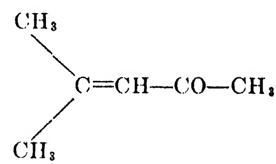
LXVIII



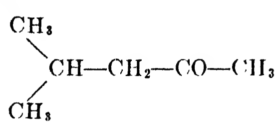
LXIX



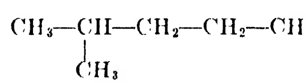
LXX



LXXI



LXXII



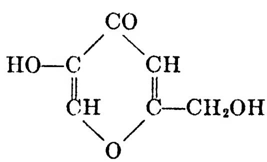
LXXIII

Another potentially important fermentation is that producing butyric acid. The process is used industrially on only a small scale at present and details have not been disclosed. Many derivatives of butyric acid are used industrially; the benzyl, methyl, octyl and terpenyl esters are used in the perfumery and essence trade and amyl butyrate, bornyl and isobornyl butyrates have been described as plasticizers for cellulose esters. Moreover vinyl butyrate is a possible ingredient of polymerizable materials. The mixed acetic and butyric acid esters of polysaccharides are also coming into favor. Cellulose acetate butyrate is marketed as an ingredient of lacquer and is less inflammable than the pure acetate. Dextran (see below) acetate butyrate may have similar uses.

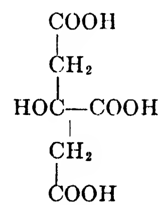
Propionic acid has been obtained by the fermentation of sucrose, as well as of many other carbohydrate materials, by a suitable strain of *Propionibacterium* in yields of about 60%. Studies have been made on

the production of propionic acid from molasses,[136–138] although no commercial exploitation of this method of propionic acid production appears to have been made. Propionic acid derivatives, however, have some industrial uses, particularly in the manufacture of perfumes and essences; probably butyl propionate is the most important. Another interesting use of propionic acid is in preventing mold growth (*Aspergillus niger*) on bread; thus if 1 lb. of calcium propionate is mixed with 400 lb. of dough, the bread can be kept for as long as ten days without molds developing.[139]

Another interesting substance, which has not however been taken up commercially, is kojic acid (LXXIV). This substance is produced with ease in about 60% yield by the growth of molds such as *Aspergillus parasiticus* on cane sugar.[140] The formulation of kojic acid as LXXIV shows it to be a compound containing many reactive centers, but despite considerable effort, particularly by Barham and Smits,[141] who have tried to make resins, dyes and drugs from kojic acid with little apparent success, no product of commercial value has been obtained from it.



LXXIV



LXXV

Another product of mold metabolism, citric acid (LXXV), is of considerable importance, and research into possible uses of its derivatives shows that it may become still more important in the future.

Citric acid, which prior to 1922 was made entirely from citrus fruits and mainly in Italy, is now produced almost exclusively by the fermentation of sucrose by means of a mold, *Aspergillus niger*. At first pure sucrose was used for this process but more recently molasses has been used instead.[142] Practically the whole of the world production of citric acid is used as such in medicinal preparations, in making soft drinks and in certain foods. The textile industries use small amounts, and it is also

(136) P. W. Wilson, Can. Pat. 302,531 (1929).
(137) J. C. Woodruff and P. W. Wilson, Can. Pat. 302,532 (1929).
(138) J. M. Sherman, U. S. Pat. 1,910,130 (1933).
(139) Ward Baking Co., British Pats., 488,560, 488,561, 488,562 (1936).
(140) J. H. Birkinshaw, J. H. V. Charles, C. H. Lilly and H. Raistrick, *Trans. Roy. Soc. London*, **220A**, 127 (1931).)
(141) H. N. Barham and B. L. Smits, *Trans. Kansas Acad. Sci.*, **37**, 91 (1934).
(142) Miles Laboratories Inc., Brit. Pat. 572,383 (1945).

used as an ingredient of engraving ink and as a silvering agent. The production of citric acid could best be stimulated by making use of certain derivatives in the plastics industry. Several esters of citric acid have been described as plasticizers and solvents and it is along this line that future research on citric acid will most probably help the sugar problem. Thus, tributyl and triamyl citrates are noninflammable liquids which plasticize cellulose nitrate and acetate and are useful solvents for spirit-soluble gums; furthermore, triethyl citrate is a good plasticizer for cellulose and phenolic resins and is doubtless now used as such. Among the resinous products obtainable from citric acid might be mentioned the alkyd resins formed with glycerol[143] and the special edible resins mentioned by Ellis[144] as being obtained from the same two materials. Some of the decomposition products of citric acid are also useful in the plastics industry. Aconitic acid (LXXVI) can be made in high yields by the catalytic dehydration of citric acid over phosphoric acid,[145] a distinct improvement on the older methods using highly corrosive acids to effect dehydration. It is also present in cane sugar

$$\mathrm{HOOC{-}CH{=}\overset{\overset{\displaystyle COOH}{|}}{C}{-}CH_2{-}COOH}$$

LXXVI

juices, and a method of isolation from alkaline earth salts has recently been published in the patent literature.[146] Esters such as triethyl, tributyl and triamyl aconitates have been described as plasticizers and a similar use for the ester of aconitic acid with 2-ethylhexanol has been suggested.[147] Kirk[148] described an interesting method of obtaining such esters by the catalytic desaturation of the monoacetates of the corresponding esters of citric acid. Little has been published on the resins obtainable from aconitic acid but clearly it is possible to make it a component of alkyd resins and such have been described by Habu.[149] The same worker in 1935 obtained a substance, which was claimed to plasticize certain resins, by heating aconitic acid with cyclohexanol.[150] Other resinous products were obtained by heating aconitic acid with terpenes (or terpene alcohols or mixtures thereof) and an unsaturated fatty acid

(143) P. I. Dmitriev, *Org. Chem. Ind. U. S. S. R.*, **6**, 111 (1939): *Chem. Abstracts*, **33**, 6987 (1939).
(144) C. Ellis, U. S. Pat. 2,007,965 (1935).
(145) R. R. Umdenstock and P. F. Bruins, *Ind. Eng. Chem.*, **37**, 963 (1945).
(146) E. K. Ventre, H. C. Henry and F. L. Gayle, U. S. Pat. 2,345,079 (1944).
(147) P. L. Gordon and Ruth Aronowitz, *Ind. Eng. Chem.*, **37**, 780 (1945).
(148) P. M. Kirk (to American Cyanamide Co.), U. S. Pat. 2,375,563 (1945).
(149) T. Habu, Japanese Pat. 93,028 (1931); *Chem. Abstracts* **26**, 4488 (1932).
(150) T. Habu, Japanese Pat. 110,730 (1935); *Chem. Abstracts* **30**, 2283 (1936).

in the presence of acid catalysts;[151] alternatively, aconitic acid could be heated first with an unsaturated fatty acid and terpene alcohol and the product esterified with a polyhydric alcohol.[152]

Citraconic acid (LXXVII) and itaconic acid (LXXVIII) are obtainable from citric acid but not so readily as is aconitic acid, although in a patent of 1936[153] there is described a method in which a strong solution

$$\begin{array}{c} CH_3\text{—}C\text{—}COOH \\ \quad\;\; \| \\ H\text{—}C\text{—}COOH \\ \text{LXXVII} \end{array} \qquad\qquad \begin{array}{c} CH_2\text{=}C\text{—}COOH \\ \quad\;\; | \\ \quad\; CH_2\text{—}COOH \\ \text{LXXVIII} \end{array}$$

of citric acid is run into an evacuated copper vessel heated at 280–300° and the anhydrides of itaconic and citraconic acids are distilled; the acids were recovered by treating the anhydrides with water. Citraconic and itaconic acids form resins when heated with a glycol and subsequently with a polymerization catalyst,[154] for citraconic acid esters themselves polymerize.[155]

Itaconic acid may be obtained by the direct fermentation of sugar solutions with *Aspergillus terreus*.[156,157] Although glucose was the sugar used, almost certainly sucrose could be used in its place. The fermentation is carried out under acid conditions so that, like the citric acid fermentation, it is one that is relatively resistant to contamination. The itaconic acid is isolated easily by crystallization of the concentrated liquors after removing the mycelium. An early attempt to form resins from itaconic acid is recorded in a patent granted to Hope in 1927,[158] who polymerized dialkyl itaconates through the agency of heat and light. Later an interesting polyester was described in an article by Garvey, Alexander, Kung and Henderson.[159] The polyester was obtained by heating together itaconic acid and ethylene glycol. It polymerizes well in films to a hard finish and is therefore of use as a drying oil. D'Alelio[160]

(151) Oelwerke Noury and van der Lande, G. m. b. H., Brit. Pat. 530,916 (1940).
(152) T. Curten (to Oelwerke Noury and van der Lande, G. m. b. H.), German Pat. 722,356 (1942).
(153) C. H. Boehringer Sohne A.-G. Brit. Pat. 452,460 (1936).
(154) C. Ellis, U. S. Pat. 2,195,362 (1940).
(155) H. B. Dykstra (to E. I. du Pont de Nemours), U. S. Pat. 1,945,307 (1934).
(156) L. B. Lockwood and G. E. Ward, *Ind. Eng. Chem.*, **37**, 405 (1945).
(157) L. B. Lockwood and G. E. N. Nelson, *Arch. Biochem.*, **10**, 365 (1946).
(158) E. Hope, Brit. Pat. 264,550 (1927).
(159) B. S. Garvey, C. H. Alexander, F. E. Kung and D. E. Henderson, *Ind. Eng. Chem.*, **33**, 1060 (1941).
(160) G. F. D'Alelio, U. S. Pats. 2,279,881–5 (1942), 2,298,039 (1943), 2,310,731 (1943).

found that certain itaconic acid esters co-polymerized with ethyl methacrylate to give harder products than are obtained when ethyl methacrylate is polymerized alone. Furthermore it was found[161] that itaconic acid co-polymerizes with acrylonitrile to give oriented fibers which could be cold-drawn into filaments of high tensile strength. Again, such itaconic acid esters as the dibenzyl and dihexyl plasticize polyvinyl chloride[162] to give products stronger than ordinary polyvinyl resins. Itaconic acid is thus becoming a resin intermediate worthy of further examination.

Among other products of fermentation of sugars, 2,3-butylene glycol (LXXIX) ranks high, and a fermentation process producing this material together with acetylmethylcarbinol (LXXX) has been developed.

$$\underset{\text{LXXIX}}{CH_3\text{—}\underset{|}{\overset{}{C}}H(OH)\text{—}CH(OH)\text{—}CH_3} \qquad \underset{\text{LXXX}}{CH_3\text{—}CO\text{—}CH(OH)\text{—}CH_3}$$

The carbohydrate material used is saccharified wheat (though sucrose also would almost certainly be a possible substrate), which is fermented with *Aerobacter aerogenes* to give a solution containing 4% of 2,3-butylene glycol and 0.2% of acetylmethylcarbinol. The importance of 2,3-butylene glycol lies in the fact that, with this compound as an intermediate, carbohydrates may be converted into synthetic rubber, since the diacetate of butylene glycol on pyrolysis gives butadiene in high yield.[163]

The derivatives[164] of butylene glycol may also be important; the ethers and esters may have uses as solvents or plasticizers; the homolog, LXXXI,[165] of ethylene oxide may have uses in synthesis, and the conversion products of butylene glycol also show promise. Neish[166] has recorded various pyrolysis products other than butadiene. Thus, when passed over a hot copper catalyst butylene glycol gives acetylmethylcarbinol, whereas when oxygen is present diacetyl is the main product. When the butylene glycol is heated with sulfuric acid, methyl ethyl ketone is formed. Butylene glycol could be used as an antifreeze material and obviously also for making alkyd resins.

(161) G. F. D'Alelio, U. S. Pat. 2,366,495 (1945).

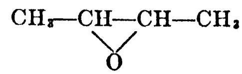
(162) G. F. D'Alelio, U. S. Pat. 2,297,290 (1942).

(163) S. A. Morell, H. H. Geller and E. C. Lathrop, *Ind. Eng. Chem.*, **37**, 877 (1945).

(164) C. H. Chappell, *Iowa State Coll. J. Sci.*, **11**, 45 (1936).

(165) M. V. Likhosherstov, L. L. Guryanova and S. V. Alekseev, *Acta Univ. Voronegiensis*, **8**, No. 2, 80 (1935).

(166) A. C. Neish, *Can. Chem. Process Inds.*, **28**, 862 (1944).



LXXXI

Glycerol is a very well known fermentation product of cane sugar. This method of obtaining glycerol was developed during the fat shortage of the first World War but since that time it seems to have been discontinued except in Germany where the method is said to have been used extensively. Since glycerol can, however, be obtained in sufficient quantities as a by-product from the soap industry and moreover may be synthesized from propylene, its production from sucrose is not likely to be developed unless greatly increased amounts are needed.

Comment has already been made upon the potentialities of lactic acid, which is obtainable from sucrose either by chemical or fermentation processes. The latter is carried out with *Lactobacillus delbrueckii* at 50° for six days using molasses as the source of sugar. The acid is neutralized as it is formed by the addition of lime. The yields of lactic acid obtained by this method are usually about 70 to 90% of the theoretical, though recently a yield of 96% has been recorded.[167]

A product of the fermentation of carbohydrates which is becoming increasingly important is fumaric acid. The efficient production of this acid by certain strains of *Rhizopus nigricans* has quite recently been realized. At the moment D-glucose or corn starch are the raw materials, but sucrose may also be used although the yield of fumaric acid is not high, as only the D-glucose portion is utilized. Several patents have been granted for processes based on the growth of *Rhizopus nigricans* on sugar solutions; for example, Kane, Finlay and Amann[168] describe a method using corn starch, D-glucose or invert sugar in the culture medium and Charles Pfizer and Co.[169] describe a similar process in which the medium is aerated during growth of the organism. Waksman[170–172] has worked extensively on this fumaric acid fermentation and has emphasized the importance of zinc salts for the growth of the organism. It has been noted that organisms other than *Rhizopus nigricans* produce fumaric acid; thus Wehmer[173] reported that a mold, which was named *Aspergillus fumaricus*, produced fumaric acid from 20% sucrose solution; Foster

(167) H. R. Stiles and L. M. Pruess, *J. Bact.*, **36**, 149 (1938).

(168) J. H. Kane, A. Finlay and P. F. Amann (to Merck and Co. and Charles Pfizer and Co.), U. S. Pat. 2,327,191 (1944).

(169) Charles Pfizer and Co., Brit. Pat. 547,594 (1942).

(170) S. A. Waksman (to Merck and Co.), Can. Pat. 414,474 (1943).

(171) S. A. Waksman (to Merck and Co.), U. S. Pat. 2,326,986 (1943).

(172) J. W. Foster and S. A. Waksman, *J. Am. Chem. Soc.*, **61**, 127 (1939).

(173) C. Wehmer, *Ber.*, **51**, 1663 (1918).

and Waksman[172] have also noted that other genera of the family *Mucoraceae,* namely *Circinella, Cunninghamella* and *Mucor,* also produce fumaric acid from sugar solutions. An article on the uses of fumaric acid by Doscher, Kane, Cragwell and Staebner[174] summarized the position up to 1941. Fumaric acid esters themselves polymerize on being heated with oxygen-yielding catalysts[175,176] to give resinous products. Fumaric acid with diethylene glycol gives an ester which polymerizes to a hard, tough resin on being heated in the presence of benzoyl peroxide. Other resins are obtained by co-polymerizing esters of fumaric acid with various polymerizable substances. Thus, dibutyl fumarate gives a rubber-like material on being co-polymerized in emulsion with isoprene.[177] Again, Hopff and Steinbrunn[178] found that rubber-like or hard products could be obtained by the interpolymerization of fumaric acid diethyl ester with isobutylene or with butadiene in the presence of peroxides. Bradley[179,180] in a patent disclosure made in 1941 describes resinous products suitable for coating or casting compositions made by heating diallyl fumarate with vinyl acetate in the presence of polymerization catalysts, and similarly resins could be obtained by heating together dipentene and dibutyl fumarate. Slagh[181] describes new resins made by co-polymerizing styrene with such esters as diallyl, dimethallyl and dicrotyl fumarate. These few examples, among the many to be found in the patent literature, indicate that fumaric acid may find extensive applications in the plastics industry.

Other acids obtainable by the fermentation of sugar are D-gluconic acid and the 2- and 5-keto-D-gluconic acids. Although these acids have been made only by the fermentation of D-glucose, indications that it would be possible to use sucrose for this purpose come from the observations of Falck, Schoeller and Michael,[182] who obtained D-gluconic acid (LXXXII) from sucrose by means of *Aspergillus niger.* Gastrock, Porges,

(174) C. K. Doscher, J. H. Kane, G. O. Cragwell and W. H. Staebner, *Ind. Eng. Chem.,* **33,** 315 (1941).

(175) H. Hopff and C. Rautenstrauch (to I. G. Farbenindustrie A.-G.), German Pat. 699,445 (1940).

(176) T. F. Bradley (to American Cyanamide Co.), U. S. Pat. 2,311,327 (1943).

(177) I. G. Farbenindustrie A.-G., Brit. Pat. 512,703 (1939).

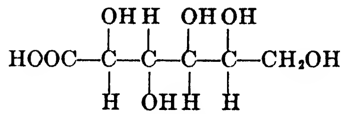
(178) H. Hopff and G. Steinbrunn (to I. G. Farbenindustrie A.-G.), U. S. Pat. 2,182,316 (1940); H. Hopff, W. Pannwitz, B. Ritzenthaler and G. Steinbrunn (to I. G. Farbenindustrie A.-G.), German Pat. 722,576 (1942).

(179) T. F. Bradley (to American Cyanamide Co.), U. S. Pat. 2,238,030 (1941).

(180) T. F. Bradley and W. B. Johnston (to American Cyanamide Co.), U. S. Pat. 2,252,393 (1941).

(181) H. R. Slagh (to Dow Chemical Co.), U. S. Pat. 2,220,855 (1941).

(182) R. Falck, W. Schoeller and S. Michael, *Biochem. Z.,* **262,** 280 (1933).



LXXXII

Wells and Moyer[183] inoculated a 20% solution with *Aspergillus niger*, carried out the fermentation with aeration in a rotary drum and obtained a 95% yield of D-gluconic acid in 24 hours; this method is probably the most efficient process for obtaining D-gluconic acid despite an attractive electrolytic method developed by Isbell.[184] Bacteria also produce D-gluconic acid, for instance *Acetobacter suboxydans*, *A. gluconicum* and *A. oxydans* all give rise to it as a product of their metabolism. At present D-gluconic acid is used mainly as a carrier for physiologically available calcium; thus calcium gluconate is used in the treatment of milk fever in cows, though recently calcium boro-gluconate has been found to be superior.[185] The amount of gluconic acid manufactured, however, is relatively small and unless new uses are found for it, it cannot materially affect the sugar utilization problem even if it were producible from sucrose.

The 2- and the 5-keto-D-gluconic acids can also be obtained by the fermentation of D-glucose, a process which almost certainly could be applied to sucrose. 2-Keto-D-gluconic acid (LXXXIII) is a product of the metabolism of certain species of *Pseudomonas*.[186,187] For instance, *Pseudomonas fluorescens*, grown on a 10% glucose solution in the presence of nutrient salts, can give a yield of over 80% of the keto acid (based on the glucose metabolized). Bernhauer and Knoblach[188] also observed that certain strains of *Acetobacter suboxydans* grown on calcium gluconate produce predominantly 2-ketogluconic acid. Chemical methods of producing 2-ketogluconic and other aldonic acids, depending on the specific catalytic oxidation of the secondary hydroxyl group at C2 in the sugar acid molecule, are also known. Thus, Pasternack and Regna[189] have described an oxidation method using sodium chlorate in the presence of vanadium pentoxide. 2-Ketogluconic acid gives rise to iso-ascorbic

(183) E. A. Gastrock, N. Porges, P. A. Wells and A. J. Moyer, *Ind. Eng. Chem.*, **30**, 782 (1938).

(184) H. S. Isbell, U. S. Pat. 1,976,731 (1934).

(185) H. T. Macpherson and J. Stewart, *Biochem. J.*, **32**, 76 (1938).

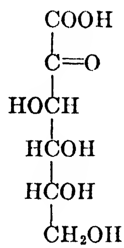
(186) L. B. Lockwood, B. Tabenkin and G. E. Ward, *J. Bact.*, **42**, 51 (1941).

(187) L. B. Lockwood, B. Tabenkin, J. J. Stubbs, G. E. Ward and E. T. Roe, U. S. Pat. 2,277,716 (1942).

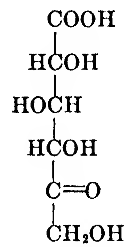
(188) K. Bernhauer and H. Knobloch, *Biochem. Z.*, **303**, 308 (1940).

(189) R. Pasternack and P. P. Regna (to Charles Pfizer and Co.), U. S. Pat. 2,203,923 (1940).

acid, an analog of ascorbic acid which has little or no antiscorbutic activity. Nevertheless, studies of the oxidation potentials of ascorbic acid and iso-ascorbic acid[190] have shown the latter to be more easily oxidizable than the former, and its incorporation into certain food products prevents loss of vitamin C by oxidation. The 5-ketogluconic acid (LXXXIV) can also be obtained by a fermentation process in excellent



LXXXIII



LXXXIV

yield. In contrast, the chemical method, which involves treatment of glucose with nitric acid, gives only a 12% yield of 5-ketogluconic acid.[191] A fermentation procedure, which involves the growing of a certain strain of *Acetobacter suboxydans* on glucose solution with aeration either in rotary drums or in vat fermenters and the isolation of the 5-ketogluconic acid as the sparingly soluble calcium salt, has been patented by Lockwood, Roe, Stubbs and Ward.[192] 5-Keto-D-gluconic acid is oxidized to L-tartaric acid by gaseous oxygen in the presence of a catalyst such as a vanadium salt, or by means of nitric acid in the presence of manganese dioxide.[193] More recently it has been found possible to carry out the oxidation under such conditions that a longer-chain dicarboxylic acid, *xylo*-trihydroxyglutaric acid (LXXXV) could be isolated in about 45% yield together with the product of further oxidation, namely L-tartaric acid.[194] Another interesting reaction of the 5-keto acid, discovered by Votoček,[195] is its conversion by acid reagents into furfural-5-carboxylic acid (LXXXVI).

(190) W. B. Esselen, Jr., J. J. Powers and R. Woodward, *Ind. Eng. Chem.*, **37,** 295 (1945).

(191) W. E. Barch, *J. Am. Chem. Soc.*, **55,** 3656 (1933).

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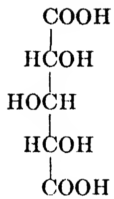

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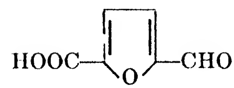

LXXXVI

Certain microorganisms produce polysaccharides when cultivated on sucrose solutions. Sugar factory "slime," for example, once regarded as a nuisance, is largely composed of the polysaccharide dextran. Dextran is produced from sucrose solutions by *Leuconostoc mesenteroides* and *Leuconostoc dextranicum* and is a polyglucose.[196,197] It can also be synthesized enzymically.[198] *Rhizobium radicicolum* produces a polysaccharide which is fibrous in character and which contains appreciable quantities of a uronic acid.[199] Levans, or polyfructoses, are also formed by the growth of certain organisms, for example *Bacillus subtilis* Cohn, on sucrose. Dextran, however, seems to be the more important from the viewpoint under discussion. Solutions of partially degraded dextran have been suggested as a substitute for plasma in blood transfusions.[200] Moreover, it may be a cheap substitute for gum arabic and gum tragacanth in certain of their applications and may well be of value as an adhesive. Another interesting use, suggested by Hamburg,[201] is as a substitute for barley malt in beer manufacture, the dextran beers being little different from malt beer in flavor and appearance. Dextran can be converted into a mixed acetate butyrate and an acetate.[202] Alkyl ethers have been prepared by Stahly and Carlson[203] by treatment of dextran with alkyl halides and sodium hydroxide and in this way the benzyl ether, which can be used in lacquers, has also been obtained.[204]

Finally some mention should be made of the production of yeast for food since this utilizes large quantities of cane sugar. At the present

(196) Frances L. Fowler, Irene K. Buckland, F. Brauns and H. Hibbert, *Can. J. Research*, **B15**, 486 (1937).

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(199) E. A. Cooper, W. D. Daker and M. Stacey, *Biochem. J.*, **32**, 1752 (1938).

(200) A. Grönwall and B. Ingelman, *Nature*, **155**, 45 (1945).

(201) M. Hamburg, *Brau- u. Malzind.*, **34**, 15 (1934).

(202) W. A. Waldie and J. E. Bersuder (to Chemical Developments Co.), U. S. Pat. 2,344,190 (1944).

(203) G. L. Stahly and W. W. Carlson (to Chemical Developments Co.), U. S. Pat. 2,344,179 (1944).

(204) G. L. Stahly and W. W. Carlson (to Chemical Developments Co.), U. S. Pat 2,380,879 (1945).

time all baker's yeast is made from sucrose, mainly from molasses; moreover this yeast production uses 24,000 tons of molasses per annum in Great Britain alone so that the worldwide use of sucrose in this way must be very considerable. Recent work at the Chemical Research Laboratory of the Department of Scientific and Industrial Research[205] has led to the development of yeasts (*Torula utilis*) which will grow under tropical conditions and which can be made into foods suitable for human consumption. Such a composition, called "food yeast" can be made cheaply, is palatable and is of high protein and vitamin B content. Another product obtainable from yeast is also of some interest, namely ergosterol. Improved cultural methods have increased the sterol content of yeast[206] so that the growing of yeast represents a method of obtaining ergosterol for vitamin D preparation. Fats also are synthesized by yeasts and other microorganisms, notably by *Endomyces vernalis*, *Oidium lactis* and *Penicillium javanicum* van Beijma. Although the amount of fat produced in some cases is considerable, the cheapness of ordinary vegetable and animal fats precludes the use of surplus sugar in this way except perhaps in the event of continued fat shortage.

VII. Utilization of Molasses

An account of work bearing on the utilization of sucrose would not be complete without some mention of the possibilities of the utilization of the residual sirups (molasses) that are produced in large amounts by all sugar factories.

The greatest outlet for molasses is of course in alcohol fermentation industries, but since alcohol can be produced from ethylene and from acetylene, which in turn can be produced from methane, research into other fermentations and other uses for molasses would seem to be expedient.

One large use for molasses is as a cattle food, used either by direct feeding or in prepared foods. The main difficulty is in handling molasses, so that the problem is to obtain it in a solid or cake form. The actual food value of molasses is high, since it contains about 55 to 60% carbohydrate material and about 4% protein. In Queensland, Kerr[207] recommends boiling the molasses with milk of lime and then pouring the mixture on a concrete floor coated with oil, so that on cooling it can be cut into cakes. Other methods of using it depend on spray-drying the molasses so that it is converted into a free-flowing powder.[208] The

(205) A. C. Thaysen, *Nature,* **151,** 406 (1943).
(206) International Yeast Co., Brit. Pat. 500,663 (1939).
(207) H. W. Kerr, *Intern. Sugar J.*, **39,** 486 (1937).
(208) Molaska Corporation, Brit. Pat. 439,595 (1935).

mixing of bagasse with molasses has also been suggested as a method of using both by-products. Sometimes, too, the molasses is returned to the earth as a manure, though this use is limited in its application by the excessive soil acidity it produces. It could, of course, be used with advantage in the reclamation of alkaline soils.

Some sugar factories, in those countries which do not possess very adequate or cheap fuel supplies, have burned molasses for this purpose. Apparently some Egyptian sugar factories have used it as a fuel for a number of years.[209] The molasses, in order to be suitable for burning, is diluted and then evaporated to 86° Brix, heated to 80°C. and filtered, to remove such impurities as might block the burners. The molasses is then injected into burners similar to fuel oil burners, but which must be made of a steel capable of withstanding the corrosive action of the molasses. The ash from this burned molasses is rich in potash and can be used with advantage as a fertilizer.

Another use for molasses is in preparing silage. In making silage, green grass or fodder is placed in large containers, compressed and allowed to undergo fermentation to such a degree that, at the end of the necessary period of storing, the fodder is preserved in a palatable and digestible condition. The soluble carbohydrates in the green fodder undergo fermentation to lactic acid, which inhibits extensive decomposition of the fodder. This lactic acid, however, can be produced by the fermentation of added carbohydrate rather than of the green fodder itself. Thus, Samurani[210] suggested adding molasses to silage; the usual amount added seems to be of the order of 15 lb. per ton of silage.[211]

Resinous materials have also been made directly from molasses; for example, Vazquez[212] describes a resin that is made by merely heating molasses with sulfuric acid and a solvent such as acetone or ethyl acetate. The extract, on evaporation, gave a hard insoluble resin which could be molded.

Indian workers have advocated the use of molasses in a polymerized form in road-making, either by direct application to a macadamized road surface or by the preparation of a molasses–bitumen composition with which granite chips may be surfaced prior to their use as road material. Hukeri[213] suggests the spreading of diluted molasses followed by sand over a road surface and claims that the surface remains dust-free throughout the dry season. Again, Sen and Frehi[214] make a mixture of

(209) H. Naus, *Intern. Sugar J.*, **40**, 141 (1938).
(210) F. Samurani, *Bureau of Agricultural Intelligence*, **5**, 1625 (1914).
(211) S. J. Watson and W. S. Ferguson, *J. Agr. Sci.*, **27**, 1 (1937).
(212) E. A. Vazquez, U. S. Pat. 1,976,590 (1934).
(213) D. H. Hukeri, *Proc. Soc. Biol. Chemists India*, **2**, 36 (1937).
(214) H. D. Sen and K. C. Frehi, *Intern. Sugar J.*, **39**, 445 (1937).

molasses with slaked lime and asphalt. Granite chips are coated with this mixture and used in the same way as ordinary tar-coated chips.

In addition to these special uses, molasses can be used for many of the processes which apply to pure sucrose. For instance, lactic acid could be made from molasses by either the chemical or the fermentation methods. Similarly citric acid can be produced by growing *Aspergillus niger* on a solution of molasses after it has been purified over a cation exchange resin whereby cations which have an adverse effect on the fermentation are removed.[142] Triggs[215] describes a pretreatment of molasses which makes it suitable for fermentation to alcohol or for yeast production. Both levulinic acid and hydroxymethylfurfural could be prepared from molasses or even from crude cane juice.[216] Stengel[217] has hydrogenated molasses mainly to propylene glycol, using as the catalyst copper hydroxide precipitated in the solution of the molasses. It is also possible to use molasses or crude cane juice as a source of nonsugar materials of value. Troitskiĭ and György[218] suggest that guanine, lecithin and adenine might be extracted from beet molasses and vitamin B_6 from cane molasses. It is perhaps opportune here to mention that the expressed cane juice itself contains a high content of the B group of vitamins,[219] so that it would be profitable and beneficial to devise methods of using a "whole sugar" in which this vitamin content is not lost.

(215) W. W. Triggs (to Standard Brands Inc.), Brit. Pat. 551,428 (1943).
(216) W. N. Haworth and L. F. Wiggins, Brit. Pats. 583,533 and 591,858 (1944).
(217) L. A. Stengel, U. S. Pat. 2,325,206 (1944).
(218) P. György, *Proc. Soc. Exptl. Biol. Med.*, **36**, 167 (1937); N. V. Troitskiĭ, Russian Pat. 42,551 (1935).
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AUTHOR INDEX*

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ERRATA

Volume 2

Page 94. Insert the ring oxygen in formula LXVI.
Page 102. In line 8 from bottom delete the dash preceding each formula.

Volume 3

Page 166. In line 5 from bottom read **α**-sedoheptitol for **α**-sedohepitol.

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